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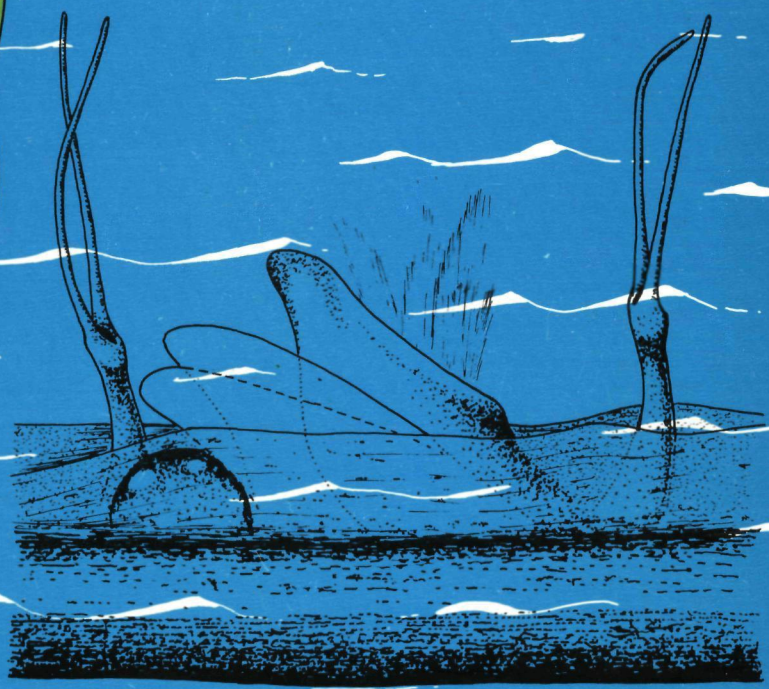
FLOWERING BIOLOGY OF THE SEAGRASS *ZOSTERA MARINA* L.



A.W.A.M. de Cock

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FLOWERING BIOLOGY OF THE SEAGRASS *ZOSTERA MARINA* L.

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FLOWERING BIOLOGY OF THE SEAGRASS *ZOSTERA MARINA* L.

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE
WISKUNDE EN NATUURWETENSCHAPPEN
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN
OP GEZAG VAN DE RECTOR MAGNIFICUS
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Aan iedereen
die op enigerlei wijze
heeft bijgedragen aan
de totstandkoming
van dit proefschrift.
Mijn hartelijke dank.

CONTENTS Pag.

INTRODUCTION

INTRODUCTION

Zostera marina L. is one of the most intensively studied seagrasses. In the history of the research of this plant, several periods may be distinguished. In the second half of the nineteenth century Grönland (1851) and Hofmeister (1852) studied the development of the flowers from the initiation of this process until flowering. Moreover, Hofmeister studied the process of fertilization. He observed pollen tubes, developing by elongation of one of the ends of the threadlike pollen grains. According to his observations, fertilization took place after self pollination. Two aspects of his study were criticized by other investigators viz. the self pollination and the way the pollen nuclei are transported to the ovule. Delpino and Ascherson (1871) noticed that flowering of *Zostera* is proterogynous, so self pollination should be excluded. Duval-Jouve (1873) described self pollination by means of the released 'fovilla' of the pollen threads. De Lanessan (1875) studied the development of the flowers also and he confirmed the observations of Hofmeister, concerning the way in which fertilization is achieved. Clavaud (1878) found a small protuberance near one of the ends of the pollen threads. This protuberance was developed when the pollen threads were released into the sea water and elongated on the stigmata to build a pollen tube. All these investigations only fragmentary dealt with flowering and pollination.

Also in later research flowering of *Zostera marina* has received little attention. After a period with decreased interest, a peak in eelgrass research may be observed in the years 1930-40. At that time investigations were concentrated on the causes, symptoms and consequences of the disastrous 'wasting disease', which destroyed in a few years nearly all North Atlantic *Zostera marina* populations. After some time the interest decreased again until in the beginning of the 1970's the attention was attracted by the considerable productivity of seagrasses. The consequence was a rise in seagrass research, which is continuing up till the present. Main subjects of study were and still are the productivity and ecology of the seagrass populations. Knowledge of flowering remained fragmentary and little detailed, although for the annual populations, which flower abundantly (Keddy and Patriquin, 1978), generative propagation is the basis of productivity. Exact knowledge of the flowering process may be a valuable help in ecological studies.

The main problem in studying flowering of *Zostera marina* is probably formed by the habitats of the plants: the intertidal zone or deeper areas, where the plants are continuously submerged. These habitats make it nearly impossible to study the course of flowering without interruption. Because of this, the first part of the present study concerned an attempt to grow *Zostera marina* plants in aquaria and to get them flowering (Chapter 1).

Observation of cultivated, flowering eelgrass plants, combined with observations in the field, made it possible to make a detailed description of the morphological development of the inflorescences from the start of flowering up to

and including the release of the seeds. (The term 'inflorescence' is used only in restricted sense: for the whole of spadix, spatha and pedunculus). A time-scale was reconstructed, that shows the relation between the subsequent stages during flowering and seed development. Attention was paid to pollination also. The release of the pollen into sea water and the transport to the stigmata as well as the consequence of pollination for the female flowers and the possibility of self pollination and fertilization have been studied (Chapter 2).

The number of different observations concerning the pollen tube development (Hofmeister, 1852; Duval-Jouve, 1873; Clavaud, 1878) was increased with one, by Harada (1957). He found that not a single but several protuberances are developed on the pollen thread in sea water. He considered these protuberances as the initiation of the pollen tubes and called this phenomenon 'primary germination'. This diversity of opinions was the motivation for a further study of the growth of the pollen tubes, by which also artifical stimulation of the pollen tube growth has been tested (Chapter 3).

Churchill and Riner (1978) and Keddy and Partriquin (1978) tried to characterize flowering of *Zostera marina* populations in the field by recording the first appearance of flowering stages (erection of the styles, dehiscence of the first anthers, release of the seeds, etc.). Since these phenomena are correlated within each individual inflorescence, this way of characterizing is similar to recording the development of the first mature inflorescence. However, flowering of a population is determined by the development of the individual fertile shoots and by the production of new shoots.

The structure of the fertile shoot has been studied by Duval-Jouve (1873), Eichler (1875) and Markgraf (1936). The way of developing in the course of time is unknown. Therefore, in the present study, the complete development of fertile shoots has been observed under controlled conditions, with daily observations. An effort has been made to characterize flowering of two different populations of eelgrass on the basis of the development of the individual shoots and the production of new shoots (Chapter 4).

Light and temperature are two factors that may play a part in the flowering process viz. in the induction of reproductive organs as well as in the diurnal course of flowering of the mature flowers. The influence of these factors on the induction of fertile shoots of *Zostera marina* has never been proved but Setchell (1922, 1929) supposed that this induction is only caused by temperatures between 15-20^{o}C and not by light. The idea, that light may act in the diurnal course of flowering came into existence when only few female flowers appeared to erect their style in the laboratory during the day. Therefore experiments were carried out to find out whether there is a day/night rhythm in flowering and if such a rhythm exists: whether it is of a persistent, endogenous nature or whether it is more or less instantly controlled by light and dark (Chapter 5).

One of the populations of eelgrass, concerned in this study, was growing in the intertidal zone, where relatively large fluctuations in temperature may occur. The conditions at low tide (for example: a cool night or much insolation) may cause large differences in temperature with the following flood. This raised the question whether temperature and temperature fluctuations influence the course of flowering. Therefore the course of flowering has been observed under three constant temperatures and two alternating temperature regimes (Chapter 6).

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1. CULTURE OF *ZOSTERA MARINA* L. IN THE LABORATORY

CULTURE OF *ZOSTERA MARINA* L IN THE LABORATORY

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ABSTRACT

De Cock, A W A M , 1977 Culture of *Zostera marina* L in the laboratory
Aquaculture, 12 279 281

It is possible to culture the seagrass *Zostera marina* L in a closed-system aquarium The conditions chosen proved to be sufficient for vegetative growth as well as flowering

INTRODUCTION

In connection with a study of the flowering biology and fertilization of *Zostera marina* L , I tried to cultivate the plants in an aquarium in the laboratory, so as to create the possibility of studying the whole course of flowering from close by I therefore chose culture conditions more or less similar to those prevailing in the natural habitat of *Z marina* during its flowering season

MATERIALS AND METHODS

Aquarium

The aquarium used was a full glass cistern of 30 x 30 x 48 cm The bottom was covered by a 4-5 cm thick layer of fine, muddy sand from the Grevelingen, a former estuary, now enclosed, in the southwestern part of The Netherlands Over the sand there was a water layer of 15 cm, with a salinity of 31 $^{o}/_{oo}$, also from the Grevelingen The aquarium was covered by a glass plate. The aquarium water could not be continuously refreshed, but instead an aquarium filter and aeration were used. The filter was filled with charcoal and filter wool Before use, the aquarium was kept in a laboratory with variable temperature (between 15 and 30°C) and light intensity for about 0 5 year Almost all the small animals living in the mud and water died during this period and rotting had stopped when seagrass was sown The aquarium was placed in a room with a 'constant' temperature of 18°C at that time. Because of the periodical illumination – 16 h light, 8 h dark – there was a daily fluctuation in the water temperature of 1-2°C During the culture period the water temperature varied between 16° and 21°C, with the exception of two periods of a few days when it was approximately 25°C These fluctuations in temperature did not have any noticeable effect on the plants

Fourteen fluorescent lamps (Philips TL M 40W/33 RS) were used for illumination. The light intensity at the water surface was about 5 500 lux. Evaporated water was replaced by distilled water.

Plants

Fruiting stems of *Z. marina* were gathered from the Grevelingen on September 5, 1974 These were kept in the laboratory at room temperature and on September 13 the spontaneously released seeds were collected. The seeds were put in an open 100-ml bottle filled with sea water and stored at 4°C in the dark. By April 10, 1975, some of the seeds had germinated at this temperature. Because of this, all seeds were sown in the aquarium. Each seed was pushed a bit into the sand.

RESULTS

The seeds already germinated died soon after transfer from bottle to aquarium. All successful seedlings were from seeds that germinated in the aquarium. On May 6, 1975, the first seedling was observed. One month later (June 6) a second one followed. Unfortunately, the first one was broken during handling and died. The second seedling developed as follows

During the first 2 months growth was rather slow but later on suddenly very fast. Between October 20 and 31 a long generative stem developed. At almost the same time the first vegetative branch was produced. The plant flowered from October 31, 1975 till January 12, 1976. Soon after seed production the whole flowering shoot died. On January 20 a second vegetative shoot was produced. The first vegetative shoot started growing faster at the beginning of February and developed a fertile stem in the first days of March. Flowering took place from March 18 to June 8. This fertile stem was also produced in a very short time 8 days ! During flowering, and later, more shoots were produced and, at time of writing (end of August 1976), the whole plant consists of more than ten shoots.

Other plants – In September 1975 three new seedlings were found in the aquarium. One of them was unintentionally half uprooted when the aquarium was replenished and did not develop further. The other two grew well, flowered from the beginning of February to the beginning of April 1976 and died soon after seed production. New shoots were not developed. On March 11, 1976, a new seedling was found which later flowered in June and July; it had developed only one other shoot by the end of August. The youngest seedling germinated on May 7, 1976, and has developed four shoots. One of these shoots produced a fertile stem and started flowering in mid-August. Whether these last seedlings developed from the old seeds (sown in April 1975 but gathered in September 1974 !) or from the seeds of the first flowering plant could not be ascertained.

ACKNOWLEDGEMENTS

I thank Professor Dr. C. den Hartog and Professor Dr. H.F. Linskens for critically reading the manuscript and correcting the English text.

2. FLOWERING, POLLINATION AND FRUITING IN *ZOSTERA MARINA* L.

FLOWERING, POLLINATION AND FRUITING IN *ZOSTERA MARINA* L.

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ABSTRACT

De Cock, A.W.A.M., 1980. Flowering, pollination and fruiting in *Zostera marina* L. *Aquat. Bot.*, 9: 201-220.

Sexual reproduction of *Zostera marina* L. has been observed in vitro. Six different stages can be distinguished: (1) the styles erect from the spadix and (2) bend back after pollination with the threadlike pollen grains; (3) pollen is released from the half anthers under water or at the water surface; (4) after maturation of 4 to 5 weeks, (5) the seeds are released from the fruit, the fruit wall remaining fixed on the spadix; (6) withering starts a few days before the release of the seeds and the flowering shoot is generally uprooted at the end of the flowering season.

The time sequence of the stages of sexual reproduction is given.

INTRODUCTION

Morphology and development of the inflorescence and fertile shoot of the seagrass *Zostera marina* L. have been thoroughly in investigated in the nineteenth century. Grönland (1851) and Hofmeister (1852) described and drew the development of the young male and female flowers. Moreover, Hofmeister described the process of fertilization which according to his observations, took place after self pollination. But the findings of these authors were criticized and supplemented by other investigators. Delpino and Ascherson (1871) noticed that flowering of *Zostera* is proterogynous, hence in contrast to the opinion of Hofmeister, self pollination should be excluded. Duval-Jouve (1853) described self pollination by means of the released 'fovilla' of the pollen threads. De Lanessan (1875) gave another extensive and illustrated description of the development of the inflorescence, flowers and embryo, and observed self pollination in a similar way to that described by Hofmeister. The study of Clavaud (1878), chiefly dealing with pollination, is the most accepted one to date. However, his article was written six years after he made his observations, so it is not surprising that it contains some incomprehensibilities and errors. Finally, Engler (1879) wrote a critical note on Hofmeister's work, without many new observations.

Phenological data on the flowering of *Zostera marina* are presented in more recent ecological publications (see Churchill and Riner, 1978, for literature). These

studies are generally restricted to field observations on the flowering period in specific areas, and on the density of flowering shoots in certain populations. Churchill and Riner (1978) also give some quantitative data on flowering and seed production in the area they examined.

The phenomena of the developmental changes during flowering have never attracted much attention, although a more detailed knowledge might be an important help in ecological studies. A possible reason for this gap may be the difficulty, if not the impossibility, of continuous observation of the development of partly-submersed flowering in the field. As a result of a successful attempt to grow *Zostera marina* plants in an aquarium (De Cock, 1977) it became possible to observe the whole life cycle of several plants under controlled conditions. The results of these laboratory observations are presented in this paper. They are supplemented and compared with field observations of flowering plants and picked inflorescences.

MATERIALS AND METHODS

Both macroscopic and microscopic observations were made of plants grown in an aquarium (De Cock, 1977), and picked flowering shoots and inflorescences. This latter material was gathered in the Grevelingen, a former estuary on the coast of The Netherlands and in the present day intertidal zone near Bergen op Zoom, The Netherlands. All plants in both these areas flowered and probably belong to the annual form, described by Keddy and Patriquin (1978). The flowering shoots were picked off and transported to the laboratory in sea water. There they were kept in glass cisterns, filled with synthetic sea water (HW-Meeressalz, H. Wiegandt, Krefeld). Temperature was kept constantly at 20°C, although a few tests were made to determine the influence of lower (15°C) and higher (25°C) temperatures on flowering. The light source was a series of fluorescent lamps (Powertube Cool White, F96T12/CW/VHO, Sylvania, Canada) with an intensity of about 14000 Lux, the light conditions being generally 12 h light and 12 h dark although the influence of continuous light and dark was also tested.

The observations in the laboratory were compared with the situation in the field. Observations about the duration of the respective stages are mainly obtained from the aquarium plants and to a lesser extent from the picked material.

All pollination experiments were done in the laboratory, using loose inflorescences from plants from Bergen op Zoom. The blade was removed for practical reasons. Full-grown, but not yet flowering, inflorescences were kept under the conditions described above until the pistils were flowering. These inflorescences, which definitely could not have been pollinated before, were then immersed in a suspension of pollen in sea water and moved to make sure that many pollen threads stuck to the stigmata. To make this suspension of pollen, some older inflorescences with flowering pistils or with pistils that already past the flowering stage, were kept under the above described conditions until dehiscence of the first anthers started.

These inflorescences were put in a small amount of synthetic sea water and kept therein for a few hours, so a dense suspension of pollen could be released.

In the various experiments, the pollinated inflorescences were kept under different conditions. The experimental temperatures were 15°C, 20°C and 25°C. The light conditions were also varied, with continuous light, continuous dark, and some lower light intensities during the period after pollination. Pollination experiments were carried out at different times of day, and cross pollinations from populations from Bergen op Zoom and the Grevelingen were also tested.

The rapidity of pollination in the sea was determined as follows: from a great number of inflorescences with anthers still present (a) the number with at least one flowering pistil and (b) the number of which all pistils past the flowering stage was counted.

Drawings were made from pictures taken with a Leica MDA camera mounted on a Leitz Elvar binocular microscope.

RESULTS

Structure of inflorescence

Though the structure of the flowering parts of *Zostera marina* has been investigated very well, there is some disagreement about the term 'inflorescence' and about the question of what has to be considered as one flower (Den Hartog, 1970). In this publication the flowers have been considered as being unisexual. The term 'inflorescence' is used here for the whole as represented in Fig. 1a.

The full-grown inflorescence of *Zostera marina* consists of a spadix, enveloped by the sheath of a short leaf, the spatha. On the flattened spadix the flowers are arranged on one side, their longitudinal axis more or less parallel to the axis of the spadix (Fig. 1d, e). The female flowers consist of an ovary with one ovule, a style and two long, thin stigmata (Fig. 1g). The male flowers consist of one anther of which the thecea are separated during the development by widening of the connective (Gronland, 1851) (Fig. 1e). The thecae are fixed to the spadix naer the apical end over about two third of their length (Fig. 1f). The spatha has its opening at the side of the spadix where the flowers are present. The margins of the spatha are very thin (two layers of cells), colourless and transparent (Fig. 1a-d) and overlap and keep the spadix closed before flowering and during the seed development.

Developmental stages

The morphological development and movements that appear during flowering did not differ in the various conditions of ligt and temperature, but the duration of the different stages of flowering was influenced by temperature and light. The picked plant material, both loose inflorescences and flowering shoots, behaved

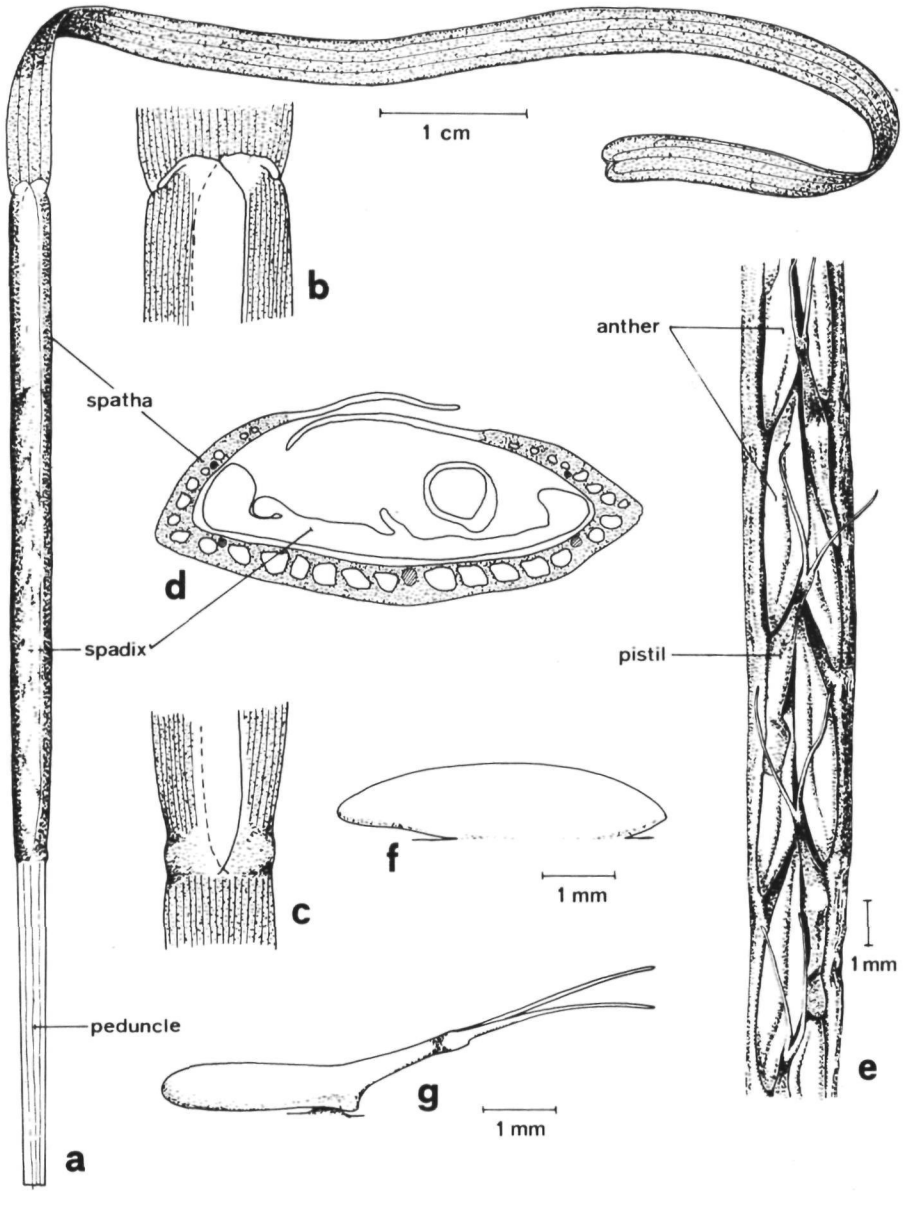

Fig. 1. *Zostera marina*: (a) complete inflorescence with peduncle, spatha, enclosing the spadix, and leaf blade; (b, c) details showing overlap of margins of the spatha (green parts dotted, transparent parts white); (d) cross section through spatha and spadix; (e) detail of spadix with male and female flowers; (f) lateral view of half-anther; (g) lateral view of female flower.

similarly during flowering to the aquarium plants. During flowering and seed development several stages could be distinguished. The classification of stages as presented below is the natural sequence by flowering in the sea.

Style erection

The first stage of anthesis is the flowering of the female flowers (Fig. 2a-c). The style of each pistil bends upward until it makes an angle of 90 degrees with the ovary and sometimes this bending goes even further. During this movement, the overlapping thin margins of the spatha are pressed apart, so that the stigmata and part of the style can project from the spatha. The pistils can be pollinated now. The styles of the pistils of one inflorescence erect one after another in acropetal sequence (Fig. 2a-c). Usually, all the styles bend upwards within a few hours and the whole process may be completed within one hour. Sometimes, however, it may take one or more days before all female flowers are in this phase, especially the one at the top of the spadix, which is often small and incompletely developed, flowering later.

It often happens that the style of this pistil stops bending at a point half way through the process and cannot get out of the spatha. The styles remain in this position until pollination takes place.

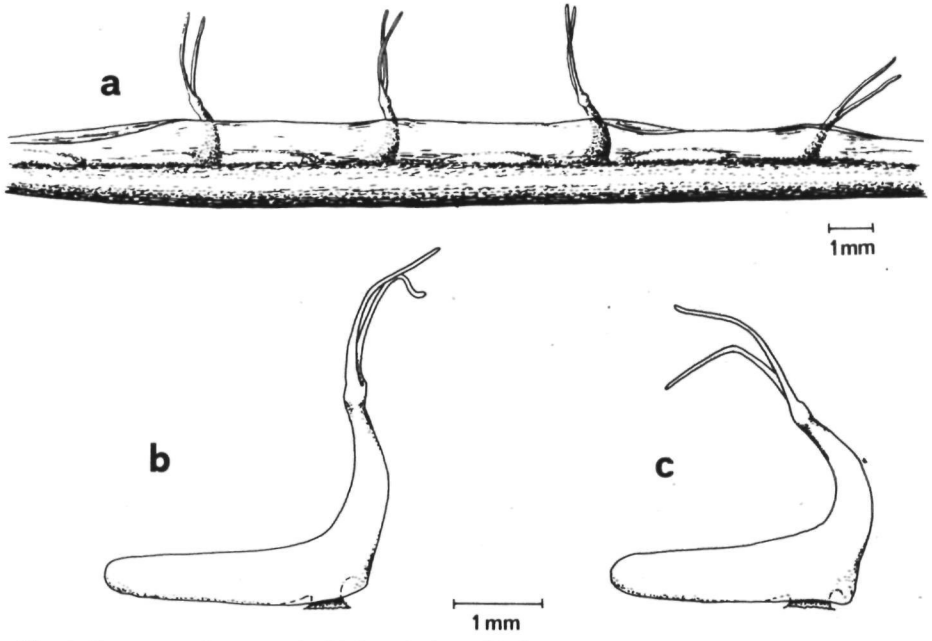

Fig. 2. *Zostera marina*, stage 1: (a) lateral view of inflorescence with erected styles; (b, c) pistils with erected styles in detail.

Back bending of the style

The second stage in the developmental sequence of the flowering inflorescence starts after pollination has taken place. As a result of pollination, the styles bend backwards until they are in the same position as before flowering (Fig. 3b and c). In the sea, this generally happens before flowering of the anthers. The start of this back-bending may be observed between 3 and 7 h after the moment of pollination. Figure 4 shows the time course of this reaction of 50 pistils in a succesful pollination experiment in which all the pistils proved to be pollinated. The experiment was carried out at 15°C. In other experiments, carried out at 20°C, the successfully pollinated styles started the process of bending backwards within 6 h after pollina-

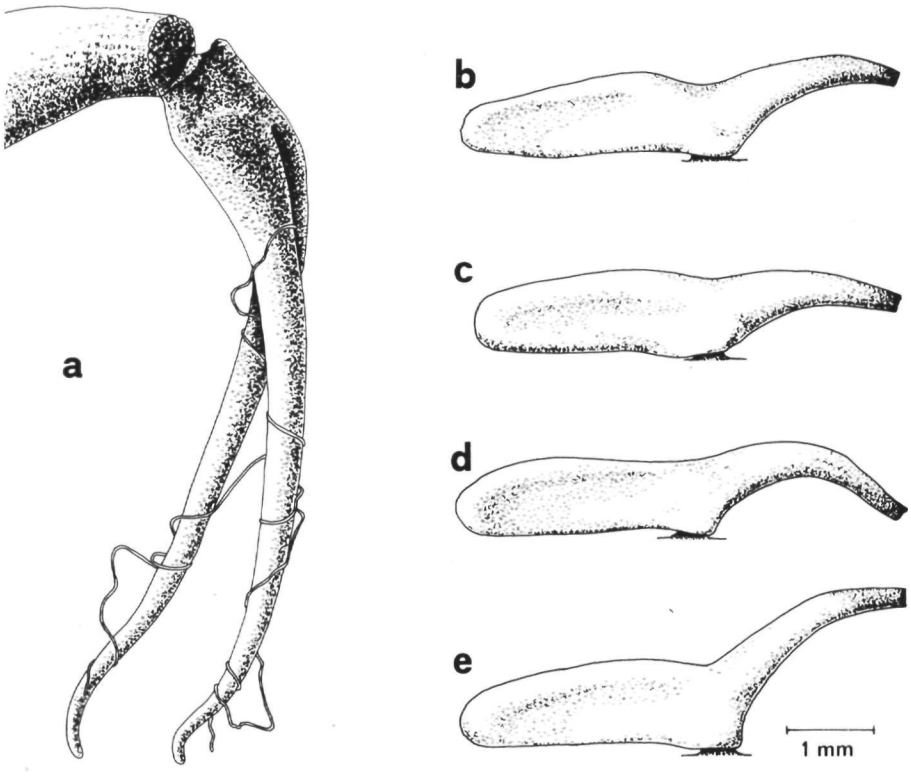

Fig. 3. *Zostera marina*, stage 2: (a) detail, showing abscission of stigmata; (b, c) pistils with styles bent back and stigmata shed; (d) pistil with strongly bent style; (e) pistil with style not completely bent back.

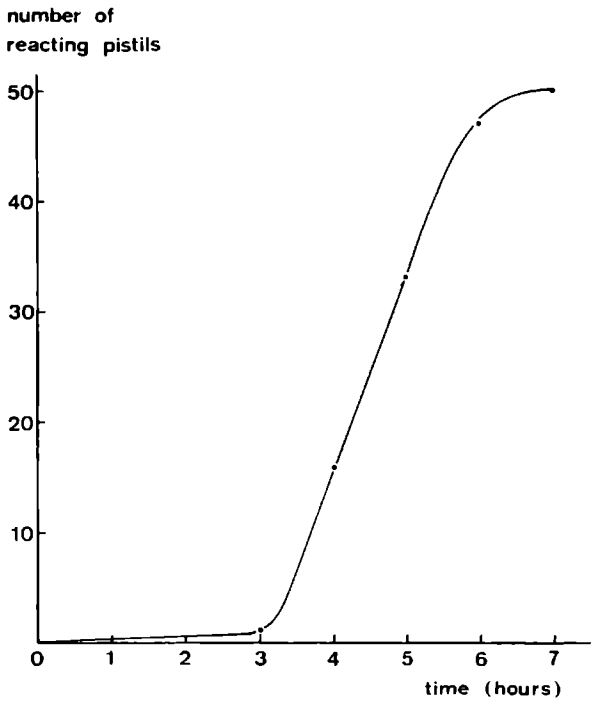

Fig. 4. Course of the process of back-bending of styles in a group of inflorescences with a total of 50 pistils.

tion. Generally, the styles come back to the inside of the spatha. The bending process may be finished within 5 h after pollination, but sometimes it may take much longer (even some days). Female flowers pollinated three or more days after the start of flowering, show this delay in particular, and their styles may even not come back to the inside of the spatha (Fig. 3e), or, on the contrary, may show an extreme degree of curvature (Fig. 3d).

Another result of pollination is the abscission of the stigmata (Fig. 3a). This takes place 7 h or more after pollination, sometimes when the style is already back inside the spatha. When all styles are back within the spatha, which normally occurs where the plants are found in their natural habitat, this stage is difficult to distinguish from the full-grown, non-flowering inflorescences. Some differences are:

(1) In the second stage the inflorescences are generally slightly darker because of a covering by epiphytes.

(2) The inflorescences are less flat and the spatha is sometimes not totally closed because of the curvature of the styles.

(3) Through the transparent margins of the spatha one may see the dark scars of the styles, where the stigmata have fallen off.

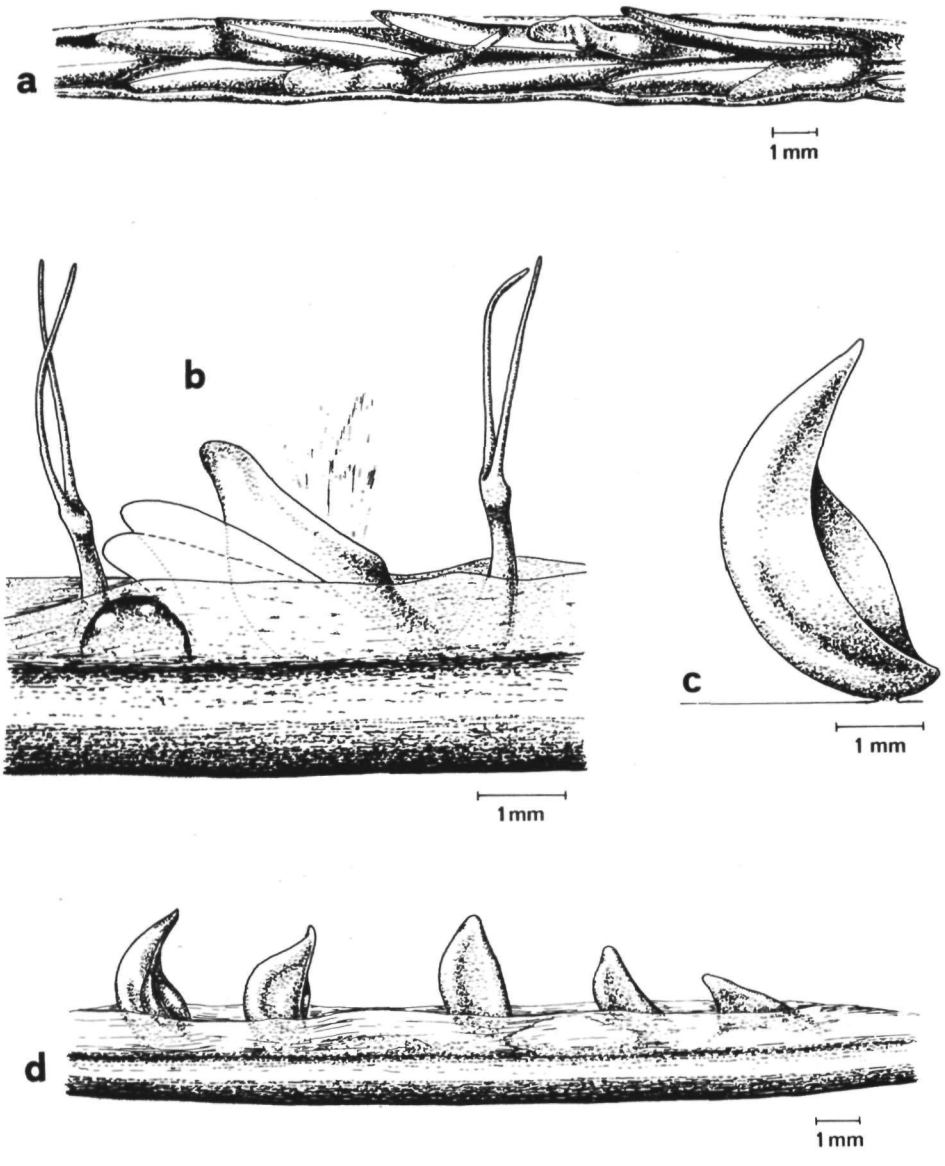

Fig. 5. *Zostera marina*, stage 3: (a) spadix with flattening thecae; (b) detail of inflorescence in lateral view (three stages of a teca, bending upwards and finally dehiscing, styles still erected); (c) empty theca wall, still fixed at a small point; (d) inflorescence, showing the acropetal way of flowering of the anthers.

The longer the inflorescence remains at this stage, the greater the differences tend to be.

If, for example in the laboratory, pollination is prevented, this second stage does not occur and the styles remain erected until the anthers of the same inflorescence flower, and self-pollination takes place. However, if even self pollination fails, the styles will not bend backwards.

Flowering of the half-anthers

The two separated thecae of one stamen do not necessarily flower at the same time. Flowering starts on average, four days after the beginning of the flowering of the pistils. Nearly all half-anthers flower between 1 and 7 days after the styles start bending upwards. This is true for both aquarium plants and picked inflorescences if kept at a temperature of 20°C. It is seldom that flowering of pistils and anthers begins at the same time, but if the inflorescences are kept at a constant temperature of 25°C for several days, this often happens.

The first sign of flowering of the anthers is the production of small gas bubbles inside the spatha (Fig. 5b). Examination with a gas analyser showed that this gas has approximately the same composition as air. In one experiment, the presence of small amounts of methane and ethylene was demonstrated. The gas is not produced if the inflorescences are kept in the dark, though flowering of the anthers in the dark normally occurs. If inflorescences with flowering anthers are transferred from dark to light, gas bubbles are produced within a few hours. If inflorescences with gas inside the spatha are kept in the dark, the gas bubbles disappear. The production of gas may be very localised, and where half-anthers occur near a gas bubble they soon start flowering. The source of the gas is most probably the point of fixation to the spadix which becomes detached at the free end of the theca. This detachment is difficult to see as it starts before the erection of the theca. However, it can be observed by adding some toluidin to the sea water, which causes the dissociated parts to turn dark blue in colour within 30 min., whereas the other parts of flowers and spadix do not absorb the dye.

At the beginning of flowering the theca becomes narrower in plan view as the sides become flatter (compare Figs. 5a and 1e). The free end starts bending upwards and the membranous margins of the spatha are pressed apart again (Fig. 5b). During this process, the fixation to the spadix becomes looser (Figs. 1f and 5c). Before the bending movement is finished, the theca begins to open dorsally at the top, along a median furrow. The margins of the theca wall curl outwards, so that the margins of the spatha are further separated, and the pollen may be released without hindrance.

The stage of the male flowering is very short. The erection of the theca may happen within one hour and usually every anther of an inflorescence flowers within a few hours till one day. The opening of the theca does not occur in an explosive way

but takes a few minutes. The theca, which starts to detach at the beginning of the flowering, finally becomes entirely loose and falls off, and the spatha closes again.

In general, the half-anthers of an inflorescence flower almost simultaneously, often, though by no means always, in acropetal sequence, as do the pistils (Fig. 5d). Sometimes only one theca of an anther erects, indicating that the flowering of two thecae that belong together is not interrelated.

Seed maturation

If pollination has taken place, a period of seed development follows. Though pollination generally occurs before flowering of the anthers of the same inflorescence, this period is referred to as the fourth developmental stage of the inflores-

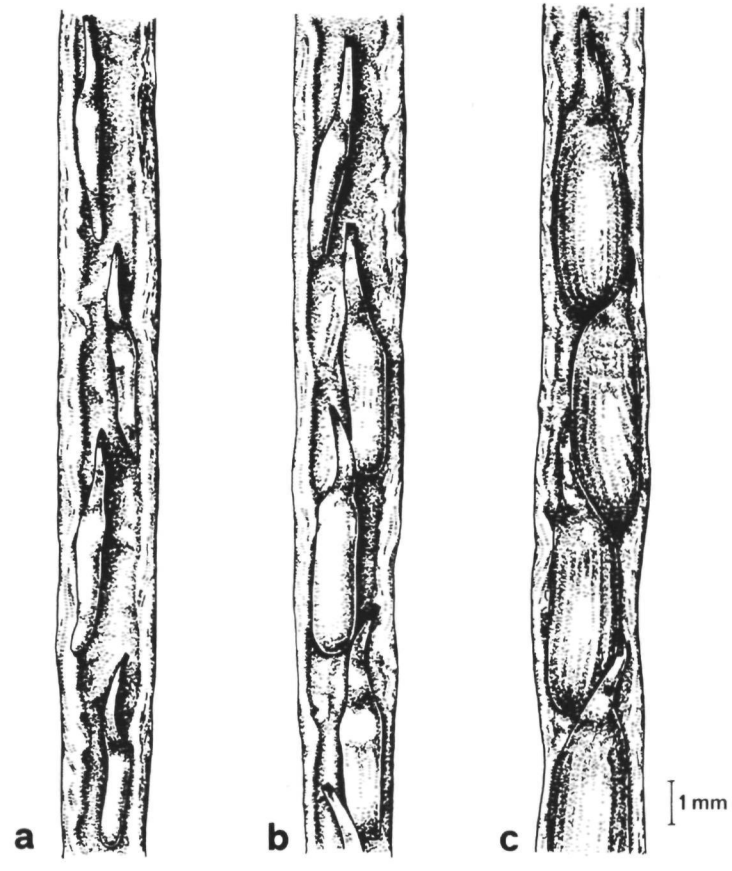

Fig. 6. *Zostera marina*, stage 4 (seed development): (a) spadix with fertilized pistils, shortly after flowering of the anthers; (b) seed development about halfway; (c) ripe fruits (striation of seeds is to be seen through the pericarp).

cence because the majority of the phase follows after the flowering of the anthers. The first macroscopical sign of the seed development is to be seen about one week after pollination. This stage can easily be distinguished since the yellowish = green anthers, clearly visible through the margins of the spatha in the previous three stages, are now absent. During the seed development, the fertilized ovule grows until it fills up the ovary; then both ovule and ovary grow further, but the style does not grow (Fig. 6a-c). The swelling of the ovary can be easily observed through the margins of the spatha. The outside of the inflorescence then becomes darker because of increasing covering by epiphytes. The total duration of the maturation of the seeds, i.e. the time between pollination and release of the seeds, was 28-38 days for the aquarium plants, the average for 19 cases examined being 32.9 days.

Seed release

When the seeds are mature, the ovary wall opens along a dorsal seam that begins at the free end of the ovary and ends at the base of the style. The margins of this opening curl outside and the margins of the spatha are separated again (Fig. 7a, b). The distal end of the fruit wall bends upward and the seed is pushed out (Fig. 7c). The seeds sink immediately because of their high specific weight. The fruit wall does not get loose but remains fixed at the spadix. The whole process of opening and seed release may take several hours and is generally finished within one day.

Mature seeds have a greyish-blue colour. This colour emanates from the cotyl, the seed coat being transparent and colourless. Many brown seeds also occur. Sometimes the testa is brown, while sometimes the testa is transparent but the contents are brown. Immature seeds are white (transparent testa, white cotyl).

The fruits of a single inflorescence do not ripen simultaneously. It was observed in the aquarium plants that pistils of the same inflorescence, pollinated at the same moment, released their seeds within a period of 6 days. Conversely, sometimes all the fruits of an inflorescence open at the same time and release mature as well as immature seeds.

In one case it was observed that an inflorescence broke off but went through every stage of flowering and seed development and finally released a normal ripe seed.

Withering

The leaf at the top of the spatha is shed during seed development and sometimes even sooner. The spatha remains green for a long time after flowering, but, particularly in the older ones, the colour is difficult to see because of the increasing cover of epiphytes. A few days prior to seed release, the green colour of the spatha begins to change to yellow. Inflorescences without fruiting pistils also finally lose their colour. This happened 26 and 37 days after the beginning of female flowering, observed in two cases in the aquarium. This corresponds to the time necessary for the ripening of fruits. The green colour gradually changes from yellowish-green, through

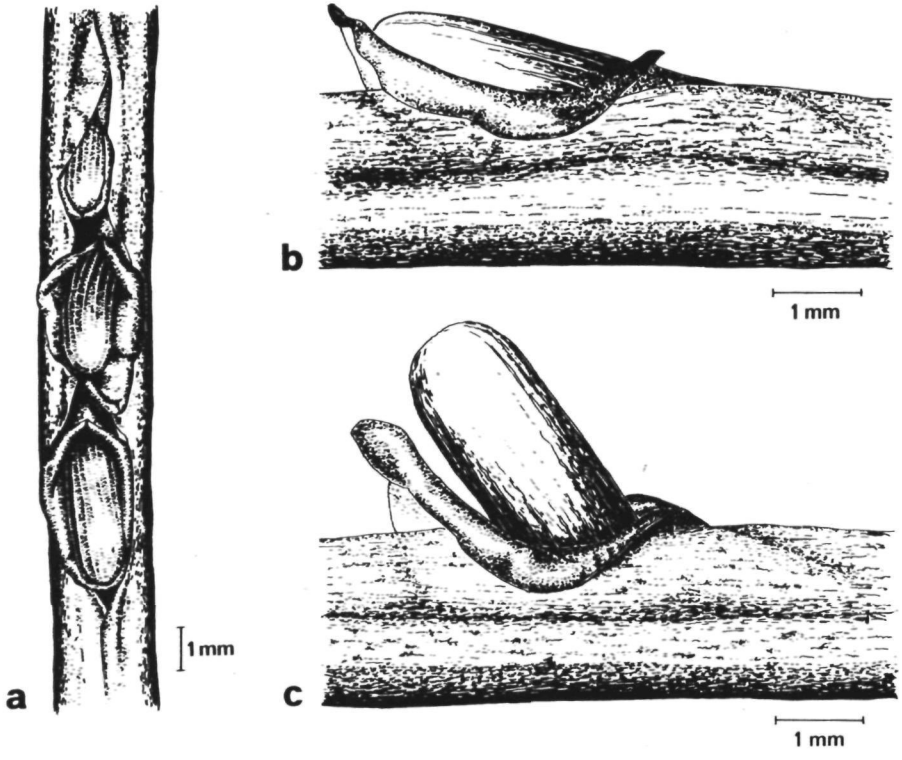

Fig. 7. *Zostera marina*, stage 5 (release of seeds): (a) inflorescence in plan view, fruits just opening; (b) inflorescence in lateral view (fruit wall curling outside, distal end of fruit starts bending upwards); (c) the fruit wall has pushed the seed out.

yellow and brown to almost black within a few weeks.

Sometimes, the spathae are shed from the peduncle some time after release of the seeds, but often they remain fixed and gradually decay. The fertile shoot and leaves turn brown too, but abscission of the leafs often occurs very early, while they are still green. The fertile shoot itself did not become detached in the aquarium plants, but gradually decayed, remaining attached to the rhizome. It proved to be impossible to remove the fertile shoots, even weeks after the last seeds were released, without uprooting the whole plant. Also, in the natural habitat, the fertile shoots remain fixed at the bottom when they are withering, though often loose fertile shoots may be found; this may be caused by heavy currents or storm waves.

The process of pollination starts with the release of the pollen. If the dehiscing theca is below the water surface, the pollen threads spread gradually through the water. As the specific weight of the pollen is a little higher than that of water, the pollen sinks slowly in stagnant water and dispersal is almost negligible if water currents are absent. If the dehiscing theca is in the surface layer of the water, for example in the intertidal zone at low tide, dispersal of the pollen takes place in another way. The pollen threads spread rapidly over the surface layer and thereby form a network (Fig. 8a) which does not sink. Further dispersal of the network is difficult, as it adheres as a whole to anything with which it comes in contact.

A factor which plays an important part in pollination is the length of life of the pollen. This was examined microscopically by counting the percentage of pollen threads with moving plasma. This length of life proved to be very different in the various anthers examined. Sometimes not one pollen thread was alive at the moment of dehiscence, and generally the number of living pollen threads was

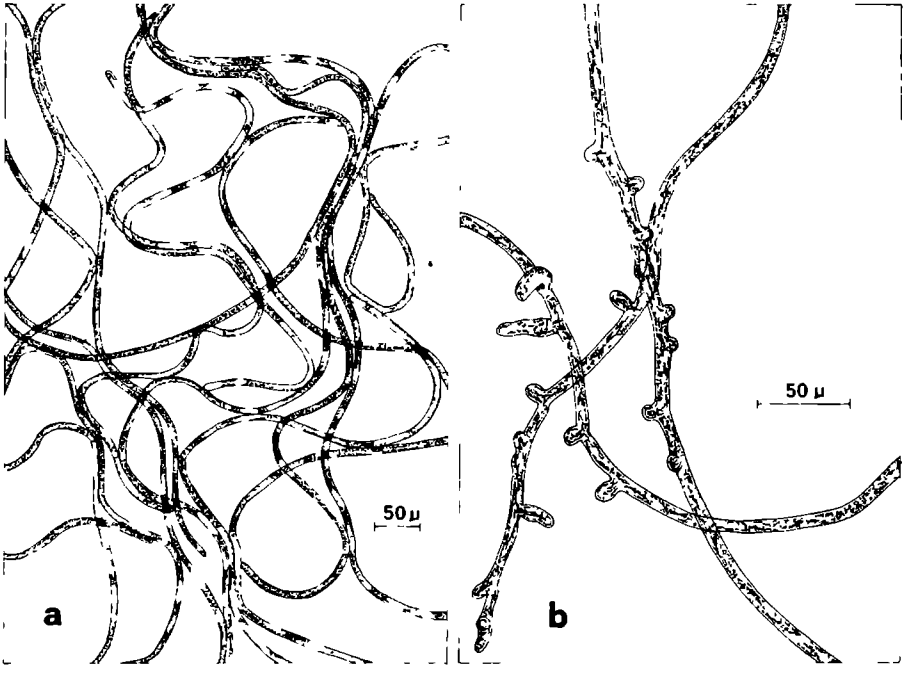

Fig. 8. *Zostera marina*, pollen: (a) network of pollen threads after dispersal at the water surface; (b) pollen threads with short pollen tubes.

reduced to 10% 20 h after dehiscence Individual pollen threads may live 48 h or longer Exact determinations were impossible because the pollen threads tended to stick to each other and to other objects For this reason, the length of life of pollen threads in the surface layer could not be determined either

The next phase in pollination is the reception by the stigmata. Active entwining of the stigmata by the pollen threads was not observed, but the pollen threads were generally to be found wound a few times round the stigmata or style Where there were many of these threads, they were hanging as a fluff on the stigmata and not every individual pollen thread reached the stigmata or style

From a great number of flowering inflorescences the numbers at stages 1 (styles erected) and 2 (styles bent back but anthers still present) were counted to determine the rate and efficiency of pollination in the sea (see Table I)

TABLE I

Ratio of numbers of inflorescences in stage 1 (female flowering) and 2 (styles bended back, anthers still present), respectively, in the intertidal zone near Bergen op Zoom

Date	No inflorescences in stage 1	No inflorescences in stage 2
9/8/77	12	124
23/8/77	3	152
4/9/77	20	150
15/9/77	36	164
25/9/77	12	129
4/10/77	19	97

In the aquarium, often only one flowering shoot at the same time was present. Nevertheless ripe, greyish blue and normal looking seeds were developed proving that effective self pollination is possible, although whether the seeds were viable could not be ascertained. Some seeds were of a transparent white and consisted of a seed coat, filled with liquid Also, loose inflorescences, kept isolated to avoid cross pollination, showed styles bending backwards after pollination by their own pollen

Many pollination experiments were carried out to discover more about the process of pollination The percentage of effectively pollinated pistils varied in identically executed experiments from 0 to 100% To find the cause of this variation, the experimental conditions were varied Different temperatures were used, experiments were carried out at different times of the day, in both light and dark, pollen suspensions were prepared in different ways and the method of pollination was varied None of these conditions had an influence such that the variability in the results could be explained

Whereas for the subtidal, perennial form of *Zostera marina* vegetative propagation is probably the most important means of reproduction, for the annual form sexual reproduction is the only way of propagation (Keddy and Patriquin, 1978). Churchill and Riner (1978) have given an interesting account of generative propagation in the sea. The present study deals with the development of the inflorescences during flowering and seed maturation.

Time sequence and duration of events

Flowering of the inflorescences of *Zostera marina* begins with erection of the styles. If no pollination occurs, flowering ends a few days later with the dehiscence of the anthers. However, in the sea pollination is a common event, as evidenced by Table I. From this table and the observations in vitro, the course of flowering in the sea can be reconstructed approximately. The beginning of the erection of the styles is at time 0. The total number of inflorescences in sea at stage 1 was 102 and at stage 2, 816, i.e. 11% and 89% of the total, respectively. If the anthers flower 4 days later than the pistils (as was the case in the aquarium at 20°C) then the styles are bent back between time points 10 and 11 h after initiation of erection (11% of 4 days). As the process of bending back may be completed within 5 h, pollination must have taken place between time points 5 and 6 h or earlier.

Back-bending of styles (stage 2) is visible at least 3 h after pollination, that is time point 8-9 h. Abscission of the stigmata occurs 7 h after pollination, at time point 12-13 h or later. The second stage of flowering lasts until the flowering of the anthers (stage 3) after 4 days. Then a long period of developing of the fruits follows (stage 4). This stage can be distinguished from stage 3 by the abscence of the anthers but strictly the development of the fruits starts at the moment of fertilization. According to Hofmeister (1852) fertilization may happen 6.5 h after pollination, about time point 12 h. Stage 4 ends at about 33 days after the start of the flowering. At that moment the fruits dehisce and the seeds are released. In Fig. 9 the reconstructed time sequence is shown. The time scale is logaritmic and the used values are approximations.

Flowering and seed development

Most striking in the first stage of flowering is the acropetal sequence in which the pistils erect their styles. This may be caused by a common stimulus that starts at the fixed end of the spadix, but probably it is the logical consequence of the acropetal way of developing of the spadix. That the pistil at the top of the spadix flowers

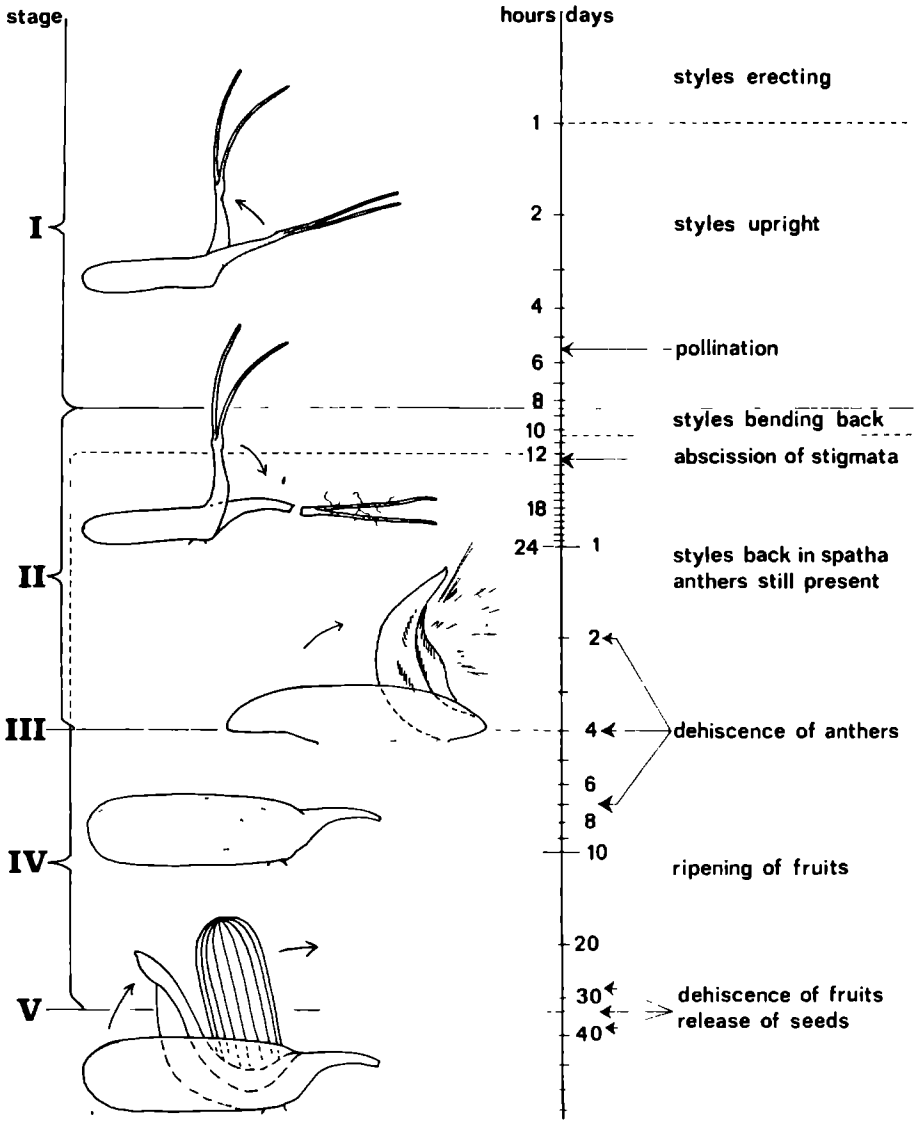

Fig. 9. Time-scale of stages during flowering and seed development of *Zostera marina*.

later is caused by purely mechanical reasons it is difficult or even impossible for the weaker developed style to pass through the margins of the spatha

The styles remain in their erect position until pollination takes place, then the second stage begins When the styles are back into the spatha, they are typically curved This curvature is not present in the young styles prior to flowering (compare Figs. 1f and 3b-d) and may indicate that growth is involved in the movement of the styles Drawings of female flowers in literature (Den Hartog, 1970, Markgraf, 1936) sometimes show this curvature and sometimes not. Because the stigmata usually fall off after the styles bent backward, it is probable that the illustrated pistils with curvature are in the second stage of flowering If the shape of female flowers of the different *Zostera* species is compared, it is necessary to indicate at which flowering stage they are A reliable comparison is only possible with female flowers of full-grown but not yet flowering inflorescences as bending backward of the styles results in different shapes of the pistils.

All styles are generally back inside the spatha before the anthers of the same inflorescence flower. Simultaneous flowering of male and female flowers is thus exceptional, but it may occur if pollination is prevented or if the plants are subjected to an extended period of high temperature The observations of Hofmeister (1852) are thus correct, but caused by abnormal conditions the plants he examined had been transported without water, during 40 h

The third stage in the development of the inflorescence is announced by production of gas bubbles This gas is produced as a consequence of an injury It is an accompanying phenomenon and does not play an important part in flowering This is supported by the fact that flowering of anthers normally occurs in the dark though then gas bubbles are not produced of even disappear Probably this gas is of the same composition as that present in the cavities of the whole plant.

More important is the presence of small amounts of ethylene Further research is needed to check whether ethylene is usually present during flowering and whether it has a function in this process. The duration of seed development corresponds to the length of time between first pollen release and first seed release in the field (Churchill and Riner, 1978) The fruits do not fall off but release their seeds. Tutin (1938) observed gas bubbles escaping when fruits dehisced. He supposed that the mechanism of dehiscence is that the ovary wall becomes weakened and is finally split by pressure of gas produced inside it by photosynthesis However, this mechanism does not explain the curling of the ovary wall and the way the seed is pushed out. According to my own observations the gas bubbles are produced by the wounded margins of the opening wall, in the same way as in the erecting thecae.

The released seeds sink immediately to the bottom. The 'buoyant, corky appendages' as mentioned by Sculthorpe (1967) were never observed. Sometimes, the seeds remain at the surface for a while, hanging on air bubbles, which does not allow dispersal over a long distance A long range dispersal is possible, however, by

means of the loosened fertile shoots. Since a loose inflorescence proved to be able to pass through the whole process of flowering and seed development, one may suppose that this may be an expedient for long range dispersal. Abscission of flowering shoots often occurs in the sea and during the four or five weeks that it takes for the seeds to ripen, such a shoot may be removed far away from the parent plant by water currents.

One can only speculate about the reasons why some seeds are released before they are ripe. From the observations that ripe seeds of one inflorescence are not necessarily released at the same moment, one can conclude that the opening of the fruits is not regulated by a common stimulus Possibly the prematurely released seeds are aborted in a late stage or the fruit is stimulated to open by external conditions.

The colours of the observed seeds are the same as those observed by Tutin (1938) brown, white and blue-grey. Churchill and Riner (1978) mention blue-grey and dark brown. According to Keddy & Patriquin (1978) the testa of the seeds of the annual form of eelgrass has a brown colour. The aquarium plants never released brown seeds, and it is therefore possible that blue-grey seeds are the normal ones. If a brown pigment in the testa does not arise during the development of the seeds, but, for example by absorption from the surroundings after the release, this colour has to be considered as not being the original one Seeds with transparent testa and brown contents are probably dead and white seeds are immature. It is therefore that only the blue-grey seeds should be used in comparative germination experiments as this might give higher percentages of germination.

Withering, in the sense of decay of the perianth, does not occur in *Zostera marina*, as the perianth is absent. The abscission of the leaf blade at the top of the spatha may be compared with the abscission of normal leaves. This appears to play no part in flowering.

The loss of colour of the inflorescences with ripe seeds and the rest of the flowering shoot may be compared with the fading of ripe corn. It is probable that abscission of flowering shoots in their natural habitats seldom occurs spontaneously. It appeared to be impossible to remove the fertile shoots in the aquarium without uprooting the whole plant Heavy water currents or possibly injury by organisms like bacteria, moulds, algae or animals seems to be necessary for uprooting or abscission of the fertile shoots in the sea

Pollination

Pollination under water is considered to be the obvious way of pollination for *Zostera marina* as most of the plants are growing below the water surface and even plants in the intertidal zone are immersed for a more or less extended period. Pollination on the surface of the water is mentioned by Kugler (1955) and Den Hartog (1970) for *Zostera marina*. Ducker et al. (1978) described this way of pollination for *Amphibolis antarctica* (Labill.) Sonder & Aschers. ex Aschers. Clavaud

(1878) described the opening of the theca as an explosive movement after which the pollen is pushed out. This is not correct. The theca opens gradually within a few minutes, and the pollen cannot be pushed out by this slow movement Under water, the pollen is dispersed by water currents and the pollen threads are separated from each other The rapid spreading of the pollen if a theca reaches the water surface may look somewhat explosive. The mechanism behind this way of dispersal is not known. Probably the pollen threads are covered by a water repelling layer so that the threads are drawn out of the water by the surface tension if a theca bores through the water surface. In this rapid reaction, the pollen threads push each other away and form a network as they make contact locally.

On the one hand this way of dispersal seems to be ineffective, as further spreading of the network is restricted as it adheres to anything it touches. Conversely, it must be realized that the opportunity for pollination is increased because both pollen and stigmata are present in the same (surface) layer at low tide The three-dimensional space for pollination under water is here reduced to a two-dimensional layer.

The threadlike shape of the pollen grains increases the floating capacity which may play a part in pollination under water. However, floating capacity is determined in the first place by the specific weight. Because of its short length of life it is unlikely that floating capacity is a significant factor A more important aspect of the threadlike shape is the greater chance for reception by the stigmata The sticky pollen adheres to anything it touches, thus also to the stigmata. The attachment is strengthened by winding of the pollen thread round the stigmata This happens in a passive way, probably by water currents and gravity I never observed active entwining as reported by Delpino and Ascherson (1871). They probably gave a misinterpretation of the observations of Hofmeister (1852).

A third significant aspect of the thread shape is enlarging of the chance that at least a part of an attached pollen thread arrives in the surroundings of the style channel This makes rapid penetration of the style possible as small pollen tubes may already be developed over the whole thread in sea water (De Cock, 1978), (Fig. 8b).

It may be concluded from the ratio of inflorescences in stage 1 and stage 2 (Table 1) that pollination in the field is very efficient. This is in agreement with the high percentage of fertile ovaries observed by Churchill and Riner (1978) Furthermore, pollination takes place very quickly if the anthers flower four days after the pistils start flowering then pollination occurs amply within 24 h A consequence is that pollination by anthers of the same inflorescence is nearly excluded. This does not prove that cross pollination is a rule because pistils may be pollinated by pollen of an other inflorescence of the same plant Effective self pollination and fertilization is possible as the plants in the aquarium proved, but to what extent this occurs in the field is not known. The case of self fertilization, described by Hofmeister (1852) was thus correctly observed, but, as mentioned before, the consequence of abnormal conditions.

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3. GERMINATION OF THE THREADLIKE POLLEN GRAINS OF THE SEAGRASS *ZOSTERA MARINA* L.

GERMINATION OF THE THREADLIKE POLLEN GRAINS OF THE SEAGRASS *ZOSTERA MARINA* L.

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Résumé. – Les filaments polliniques de *Zostera marina* L. paraissent germer avec de petites protubérances (5-10 µ en moyenne) dans l'eau de mer pure ou diluée à 75% et 50%, mais la germination est rare dans l'eau de mer artificielle. Des tubes polliniques de plus de 100 µ n'ont été trouvés que sur les stigmates. Seuls les milieux à 100%, 75% et 50% d'eau de mer, additionnés de 10% de saccharose et d'extraits de pistils ont montré un effet stimulant sur la germination et l'allongement des tubes polliniques.

Summary. – The threadlike pollen grains of *Zostera marina* L. appeared to germinate with short protuberances (average 5-10 µm) in natural sea water and its dilutions of 75% and 50%, but seldom in artificial sea water. Very long pollen tubes of more than 100 µm were only observed on the stigmata. From the tested media only 100%, 75% and 50% sea water with 10% saccharose and extract from pistils showed a stimulating effect on pollen tube germination and growth.

INTRODUCTION

In 1852 Hofmeister investigated the fertilization in the eelgrass *Zostera marina* L. He supposed that the pollen tube arose from one end of the threadlike pollen grains. Duval-Jouve (1873) could not find any pollen tube at all and thought fertilization takes place by way of the released plasma. The first who observed short protuberances on the pollen threads was Clavaud (1878). He saw near one end of each pollen thread one branch of 2-3 times its diameter, already developed in sea water. Harada (1957) observed several branches on one pollen thread. According to him these protuberances are developed within 10 minutes as a result of a reaction with sea water and not owing to contact with stigmata. He called this the 'primary germination'. He saw no further elongation of the pollen tube (which he called 'secundary germination') and did not notice any effect of osmotic pressure, pH, sugar and organic acids.

In the present study, germination on the stigmata and in sea water was examined microscopically and the effect of several media on germination was tested.

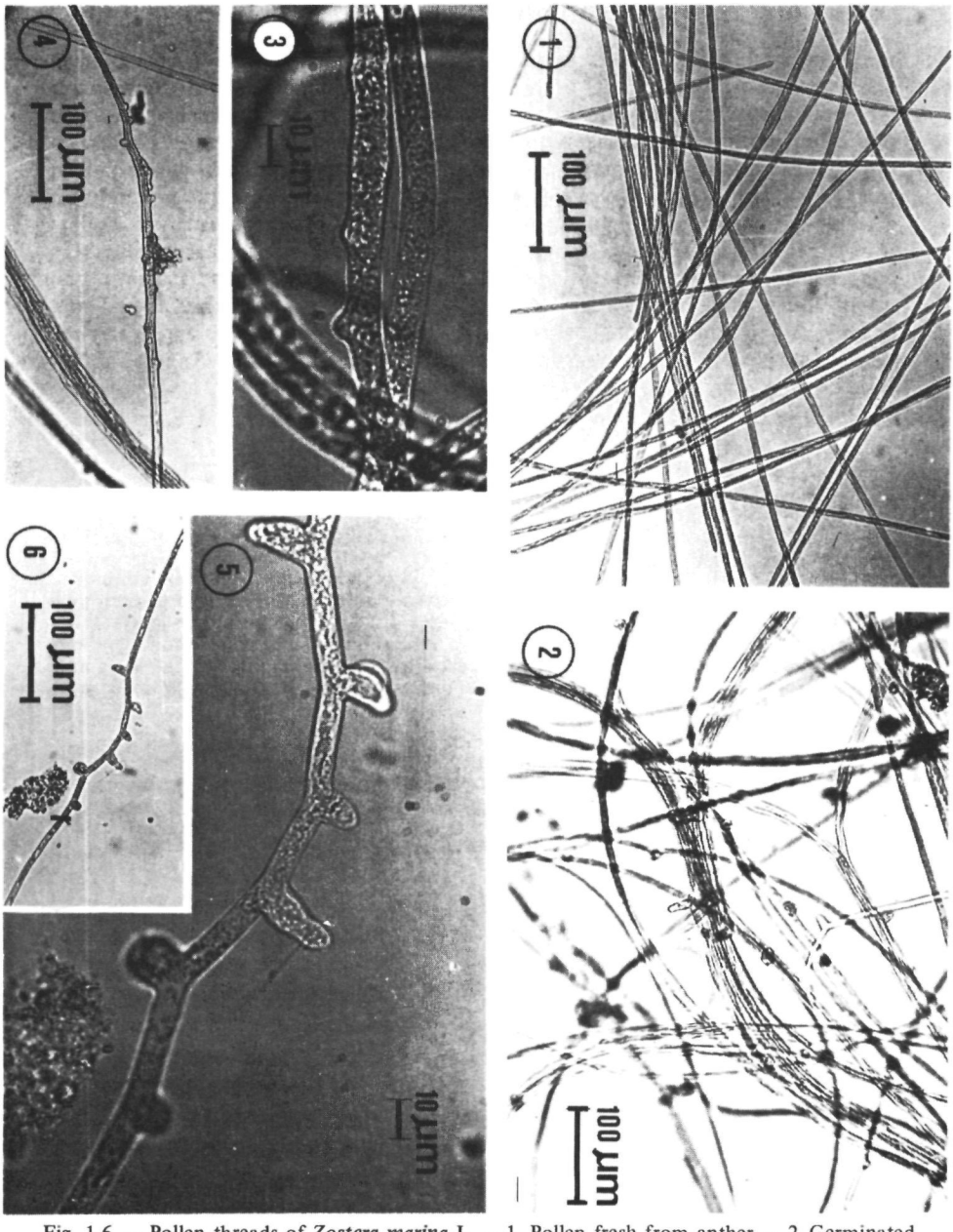

Fig. 1-6. – Pollen threads of *Zostera marina* L. – 1. Pollen fresh from anther. – 2. Germinated pollen threads. – 3-4. 'Primary germination' in control 100% sea water. – 5-6. 'Secundary germination' in medium 50% sea water with 10% saccharose and extract of pistils.

MATERIALS AND METHODS

Flowering *Zostera marina* L plants were gathered in Bergen op Zoom (The Netherlands) and in Roscoff (France) Mature, closed anthers were removed from the flowering inflorescences The anthers were opened in the test media with two needles Stigmata of pollinated pistils were cut off and looked at microscopically

The media with pollen were kept in closed dishes at a temperature of 18-20°C After three hours of incubation the results were observed. Tested media were pure sea water (= 100%) and dilutions of 75%, 50% and 25% sea water. 10% of saccharose and an extract of the pistils was added. The extract was made by grinding pistils with sea water (one pistil in one ml. of sea water) in a mortar and filtrating. 2 1/2 ml. of this filtrate was added to 100 ml. of (diluted) sea water In Roscoff I used natural sea water and dilutions with tap water for the test media The pollen of Bergen op Zoom were treated with artificial sea water (HW-Meeressalz, H. Wiegandt, 415 Krefeld 1) and dilutions with distilled water The controls consisted of the same dilutions of sea water without additions.

RESULTS

Almost all stigmata observed were covered with pollen threads of which at least a few showed one or more pollen tubes of different length, in general not exceeding several tens of microns Very seldom a pollen tube was observed longer than 100 microns. Pollen tubes were developed over the whole thread or grouped in the middle or near one end. The number of pollen tubes ranged from 1 to about 20 per pollen thread The pollen tubes had about the same diameter as the pollen threads, 8-10 µm. Sometimes plasma was seen flowing out of the tips of the pollen tubes. Germination pores were not seen.

Germination was seldom seen in pure, artifical sea water nor in its dilutions or in 25% natural sea water Short pollen tubes of 5-10 µm were developed in 50%, 75% and 100% natural sea water (dilutions with tap water) (Fig. 3-4) The media consisting of 50-100% both natural and artificial sea water with saccharose and extract of pistils gave a clear stimulation of germination (in artificial sea water) and growth of pollen tubes (in natural sea water) (Fig. 5-6).

In the tested media with saccharose (10%) with boric acid (0,1 resp. 0,01%) or yeast extract (1%) no germination was observed. Results of the germination tests are given in Table 1. Since the very long pollen threads were sticking together in an inextricable tangle it was impossible to make a reliable determination of the percentage of germinated pollen threads Therefore only the length of pollen tubes was used to determine stimulating effects of the tested media.

TABLE 1

Average and maximum length of pollen tubes in germination tests.

The data from tests with pollen from Bergen op Zoom are exact measurements but the data from the pollen from Roscoff are estimations. All data in microns. n.t. = not tested; * = in this test only 5 germinated grains were seen.

	Pollen from Bergen op Zoom (in artificial sea water)		Pollen from Roscoff (in natural sea water)	
% sea water	control	+ sacch. + extract	control	+ sacch. + extract
25%	0	8 (max. 13)*	0	n.t.
50%	0	10 (max. 30)	5-10 (max. 30)	30 (max. 50)
75%	0	12 (max. 32)	5-10 (max. 30)	30 (max. 50)
100%	0	8 (max. 19)	5-10 (max. 30)	n.t.

DISCUSSION

The germination of the pollen threads in natural sea water and its dilutions with tap water and the negligible number of germinated pollen threads in artificial sea water and its dilutions with distilled water suggest that a mineral compound of the sea water is not responsible for the 'primary germination'. It is more likely that an organic substance, only present in natural sea water, induces germination. One can speculate that it may diffuse out of the stigmata so that activation of the pollen threads takes place in the vicinity of the pistils. There might also be an influence of the mineral substances in tap water.

As the differences between 'primary germination' in sea water and 'secundary germination' (elongation of the pollen tube in the test media and on the stigmata) consist of differences in length of the pollen tubes only, I suggest that this is only a relative difference, due to the lack in sea water of necessary substances for further development of the pollen tube.

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4. DEVELOPMENT OF THE FLOWERING SHOOT OF *ZOSTERA MARINA* L. UNDER CONTROLLED CONDITIONS IN COMPARISON TO THE DEVELOPMENT IN TWO DIFFERENT NATURAL HABITATS IN THE NETHERLANDS

DEVELOPMENT OF THE FLOWERING SHOOT OF *ZOSTERA MARINA* L. UNDER CONTROLLED CONDITIONS IN COMPARISON TO THE DEVELOPMENT IN TWO DIFFERENT NATURAL HABITATS IN THE NETHERLANDS

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ABSTRACT

De Cock, A.W.A.M., 1981 Development of the flowering shoot of *Zostera marina* L. under controlled conditions in comparison to the development in two different natural habitats in The Netherlands. Aquat. Bot. (in press).

The development of flowering shoots was studied by daily observing plants, grown in aquaria under controlled conditions. Inflorescences ('spathes') of a rhipidium develop and flower one after an other, thus elongating the fertile shoot. Rhipidia of the same fertile shoot flower more or less synchronously The whole course of flowering of one generative shoot is given.

There is no peak in flowering in the field as a consequence of the long time that a fertile shoot flowers. In the intertidal zone near Bergen op Zoom (The Netherlands) the production of new shoots increases up till the end of August and then gradually declines. In the beginning of October the development of new fertile shoots stops and at the end of this month nearly all plants are withering or washed away.

In the stagnant water of the Grevelingen (The Netherlands) the production of fertile shoots increases in July, in constant or slightly fluctuating in August and suddenly stops in the beginning of September. The flowering rate is somewhat higher in the Grevelingen than near Bergen op Zoom.

INTRODUCTION

For many of the populations of eelgrass, *Zostera marina* L., in The Netherlands, the generative way of propagation is by far the most important. Almost every plant develops one or more fertile shoots. The factors inducing formation of fertile shoots are not known, but from investigations of the seagrasses *Halophila engelmanni* Aschers., *Cymodocea rotundata* Ehrenb. & Hempr. ex Aschers. and *Syringodium filiforme* Kutz. it is known that temperature and most probably also daylength play a part (McMillan, 1976, 1979 and 1980). A diurnal rhythm of 16 hours light/8 hours dark at a temperature of about 20°C appeared to be suitable for development of fertile shoots of *Zostera marina* (De Cock, 1977).

The structure of the fertile shoot has been described very well. One of the

first extensive descriptions is that by Duval-Jouve (1873). Eichler (1875) has described the sympodial development of the rhipidium and illustrated this description with a clear schematic drawing. The most complete description of the whole generative shoot, including illustrations, has been made by Markgraf (1936). Setchell (1929) has published data on the phenology of the perennial *Zostera marina*. According to this author these plants did not flower in the first year of their development. Fertile shoots were developed between 10-15°C but they did not flower within this temperature range. The phenology of the annual eelgrass has been described by Keddy & Patriquin (1978). They gave a table with 10 'stages in the sequence of flowering shoot development' and tried to characterize the flowering in the field by using the first records of these stages as parameters. Churchill & Riner (1978) also used some of these parameters in their study of flowering of eelgrass in Great South Bay.

However, these parameters are correlated within each separate inflorescence (see definition below). Recording the first appearance of mature flowers, flowering of female and male flowers, seed development and seed release, will be similar to recording the development of the first mature inflorescence of the first mature fertile shoot. The course of flowering of a population in the field is determined by the development of the individual shoots but also by the development of new fertile shoots. External factors (temperature, light, nutrient supply) will influence both these processes.

In this study the complete development of individual flowering shoots was observed under controlled conditions. An effort has been made to characterize the course of flowering of 2 populations of different habitats on the basis of the development of the individual shoots and the production of new shoots.

Definition of inflorescence. The term 'inflorescence' has been used in the literature about *Zostera marina* both for the whole generative shoot and for the spadix, enclosed by its spatha (Den Hartog, 1970). In the last case it is often called 'spathe' (Den Hartog, 1970; Churchill & Riner, 1978; Keddy & Patriquin, 1978; Jacobs & Pierson, 1981). However, considering the flowering parts of *Zostera marina*, one may recognize a flowering unit at three levels (Fig. 1):

a. The whole generative shoot. The generative shoot may be branched several times and every branch alternates with a normal, vegetative leaf. Lanjouw (1968) defines 'inflorescence' as a cluster of two or more flowers on branches, not separated by normal leaves. According to this definition the term 'inflorescence' may not even correctly be applied to the branched fertile shoot. In the present paper I have used the terms 'generative shoot', 'fertile shoot' or 'flowering shoot' for this unit.

b The rhipidium. This is a compound inflorescence, consisting of several spadices. The term 'branch', as used by Churchill & Riner (1978) has to be avoided for this flowering unit, as a flowering shoot may be branched more than once, so a branch may consist of more than one rhipidium. In this paper this unit is called 'rhipidium'.

c. The 'inflorescence' in restricted sense ('spathe'). The smallest flowering unit is the spadix, including spatha and peduncle. The spadix is completely enveloped by the spatha. Probably for this reason this unit is generally called 'spathe'. However, one might say that a 'rhipidium consists of several spathes', but it is not correct to talk about 'flowering spathes'. Because of this I have used the term 'inflorescence' in restricted sense for this smallest flowering unit only and the term 'spathe' (spatha) if the spathal sheath and leaf are meant.

LOCALITIES AND METHODS

Observations were made on plants of *Zostera marina* which were grown from seeds and flowered in aquaria in a light/dark regime of 16/8 h at a temperature of about 20°C (De Cock, 1977). The development of several fertile shoots was followed by frequent observations; data on the state of flowering of any of the inflorescences of one special shoot were collected daily.

To know more about the course of flowering in the field, two populations from different habitats in The Netherlands were studied:

a. A population growing in the intertidal zone near Bergen op Zoom. The plants are wholly submerged at high tide but at low tide they are standing in pools of a few centimetres depth.

b. A population in the stagnant water of the Grevelingen, a former estuary, where the plants occur submerged. Observations were made in a part of this lake, where the water is 40-50 cm. deep. The plants in this population have fertile shoots that grow to such an extent that the main part is floating on the water surface. The plants of both these populations are annual and have the same habit; however, the plants of the Grevelingen are more yellowish green.

At each site field observations were made. A number of flowering shoots was collected in both areas every 1-2 weeks during the flowering season of 1977. At least 250 ripidia of each collection were used for examination of the flowering stage.

RESULTS

1. General observations

The flowering process in *Zostera marina* is initiated by the development of a long generative shoot from an originally vegetative shoot (Tomlinson, 1974). The internodes start to elongate and are visible before one can see the inflorescences macroscopically and without preparation. The generative shoot may be branched one or more times and at its end and at the ends of the branches, the inflorescences arise (Fig. 1).

The inflorescences do not start flowering all at the same time. Per terminal

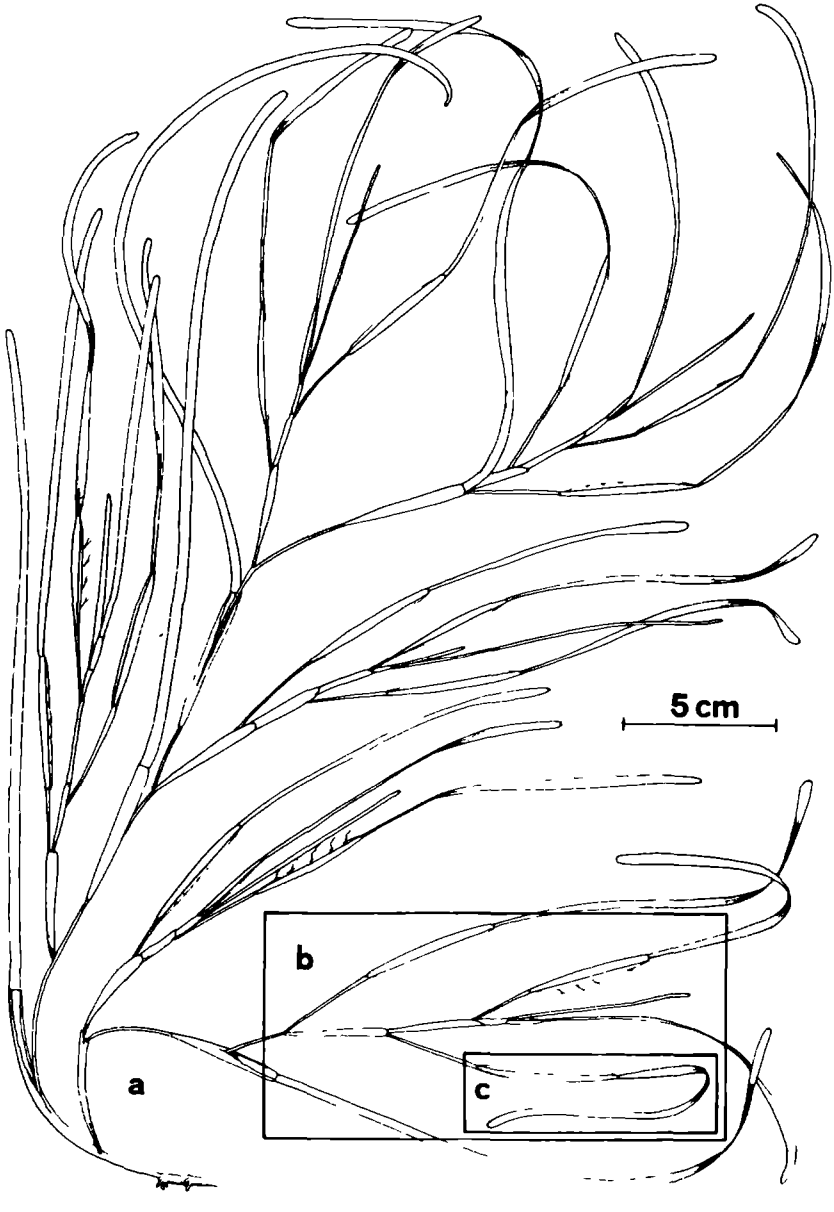


Fig. 1. *Zostera marina* L. Flowering shoot (a), rhipidium (b) and inflorescence (c).

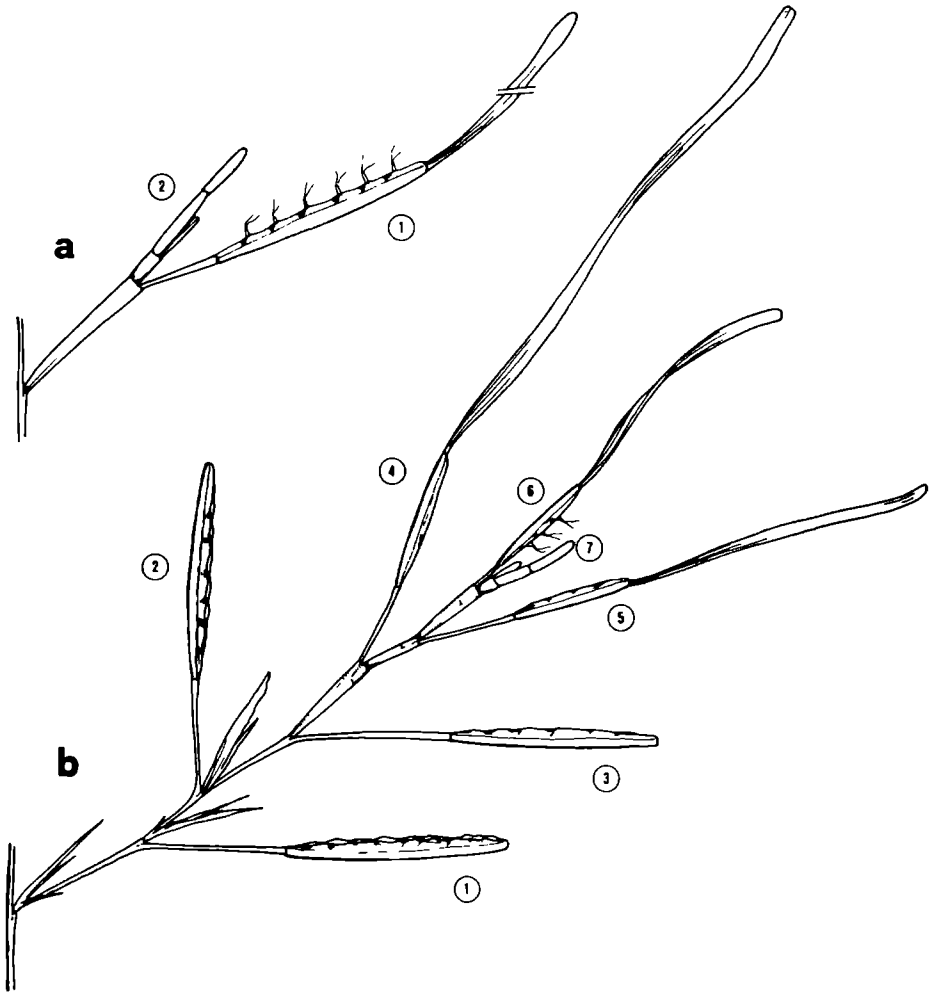


Fig. 2. *Zostera marina* L. Rhipidia in two stages of development.

a. Young rhipidium with first inflorescence flowering; second one developing.

b. Rhipidium near the end of its flowering period: prophyllum and spathal blade of the older inflorescences are withering or broken off. Inflorescence 7 still developing, number 6 flowering with styles projected and the rest off flowering.

Numbering of inflorescences according to their order of development and flowering.

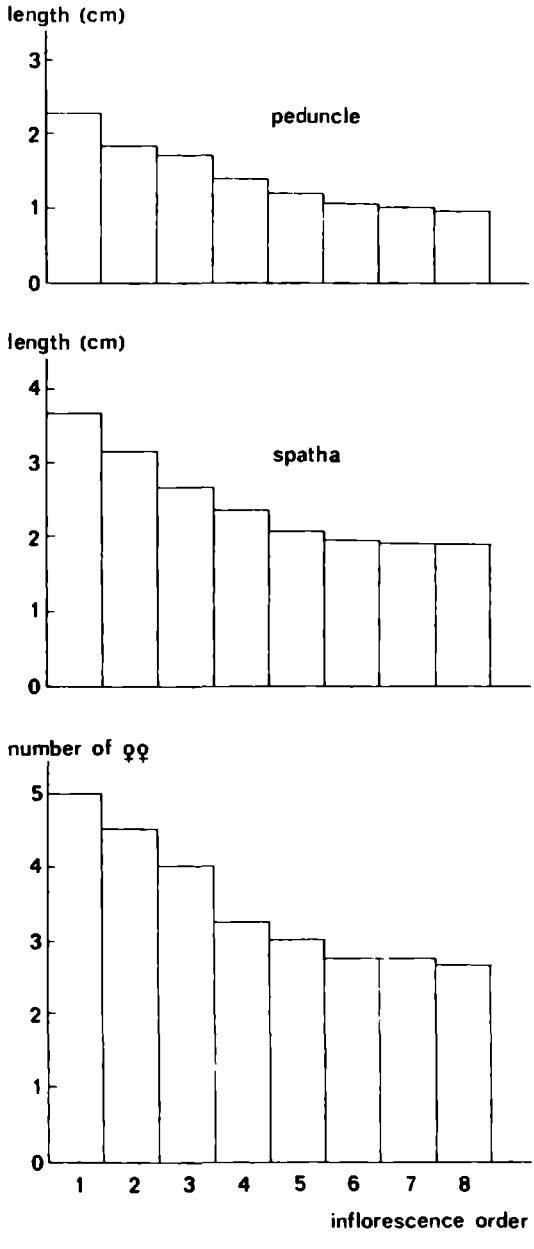


Fig. 3. Number of female flowers, length of peduncle and length of spatha with increasing order number of inflorescence. Data of the flowering shoot of an aquariumplant.

branch only one inflorescence is flowering at the same time; at that time the next one of the same branch is not yet entirely developed (Fig. 2a). The second inflorescence starts flowering a few days after the first one opened its anthers. During the season the inflorescences develop one after an other in the way described and compose a rhipidium (Fig. 2b). So it seems able to flower indefinitely.

However, flowering stops after some time, even when the flowering season is not yet past and the external conditions seem to be still favourable for flowering. The plants in the aquarium did not develop more than 6-9 inflorescences per riphidium and the number of inflorescences developed in the seagrass beds near Bergen op Zoom and in the Grevelingen was about the same.

A remarkable phenomenon, considering single rhipidia, is the decreasing size of the successive inflorescences. If the inflorescences are numbered in the sequence of development, then the length of peduncle and spatha as well as the number of flowers per spadix generally decrease with increasing order number. This was particularly clear in the aquarium plants (Fig. 3).

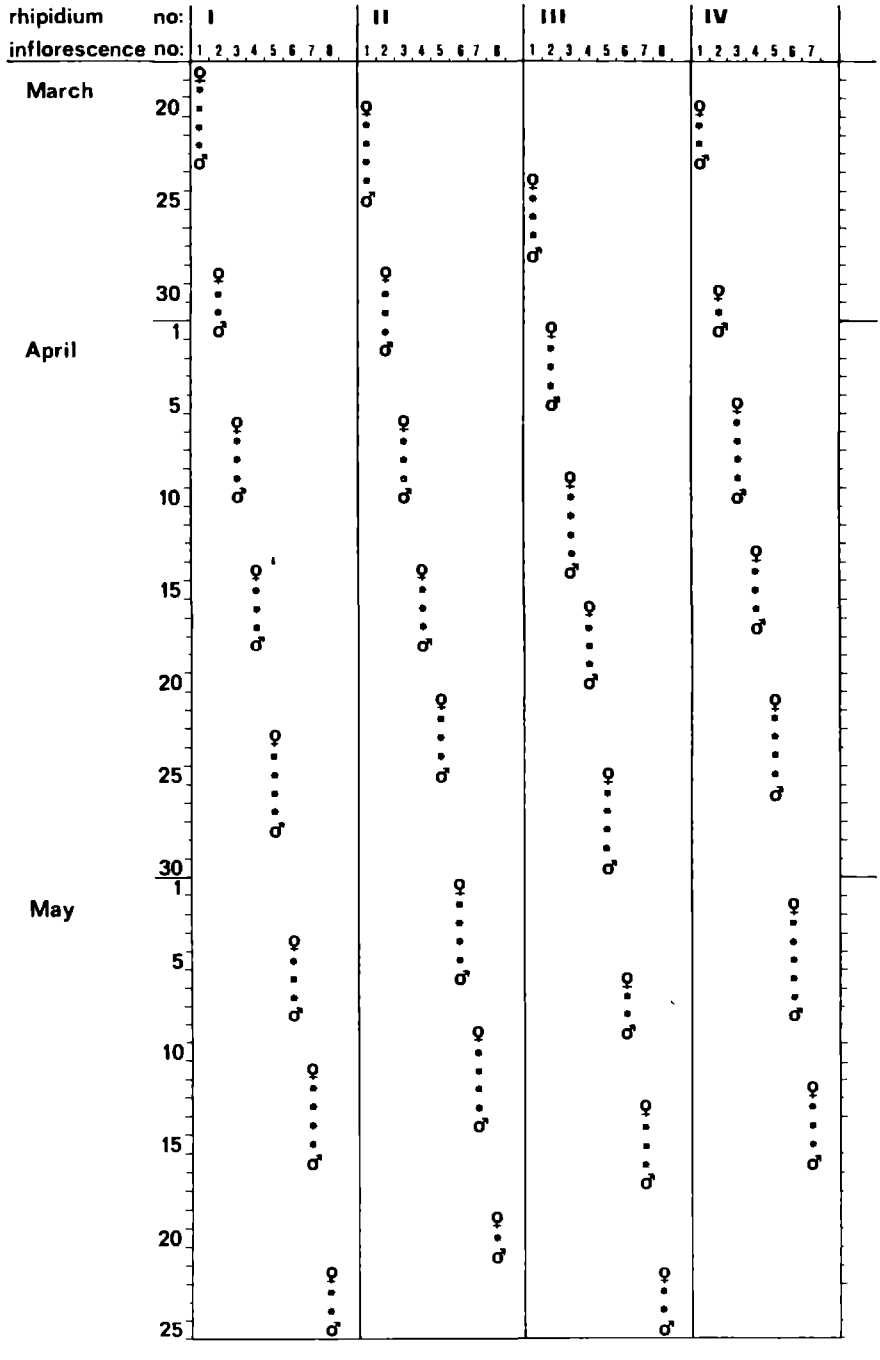
As mentioned before, seldom two inflorescences of one rhipidium flower at the same time but flowering of the rhipidia of the same generative shoot is more or less synchronous. Especially if the shoot is small and little branched, its rhipidia are in about the same state of flowering. In large flowering shoots greater differences in the state of flowering of the rhipidia may occur.

2. Development of the flowering shoots under laboratory conditions

In the aquaria, the plants developed after germination one or more vegetative shoots. The oldest shoot developed into a generative one after 3.5-5 months. One plant produced two fertile shoots: the first one 145 days after germination and the second one 277 days after germination. The difference of time between the first macroscopically visible appearance of the fertile shoot and the flowering of the first inflorescence was only 6-9 days in 5 cases examined. In that time the fertile shoot reached a length of 15-40 cm. The fertile shoot had its main growth in the initial period and further elongation took place because of the development of the rhipidia.

Each shoot produced 1 to 4 rhipidia. In its turn each rhipidium produced 6-9 inflorescences, although a number of 7 or 8 was most common. The whole course of flowering of a generative shoot that may be considered as being representative for the flowering shoots in the aquaria, is shown in Fig. 4. The rhipidia developed rather regularly. Nearly always there was a time lapse of one or more days between the flowering of two successive inflorescences: that is between the dehiscence of the anthers of one inflorescence and projecting of the pistils of the next one. The period of flowering of the female flowers was extended until pollination took place. In Fig. 4 only the start of this period is indicated.

Flowering of the 4 rhipidia was more or less synchronous; deviations were


rhipidium no: I II III IV
inflorescence no: 1 2 3 4 5 6 7 8
March
April
May
20
25
30
1
5
10
15
20
25
30
1
5
10
15
20
25

never more than 1 order number. Average length of peduncle and spatha and the number of pistils of the successive inflorescences of this shoot are given in Fig. 3.

The orientation of the inflorescences with regard to the surface of the water was as follows. In the beginning, during the development of the first 4-5 inflorescences, the flowering shoot was floating in such a way that the opening of the spathae was in turn to above and to below. When the number of inflorescences increased, this orientation became gradually less obvious and finally the rhipidia were floating on the surface in a fan shape with every inflorescence with its lateral side to the surface of the water. Of course this happened only if a fertile shoot could reach the water surface. Below the water no special orientation was found.

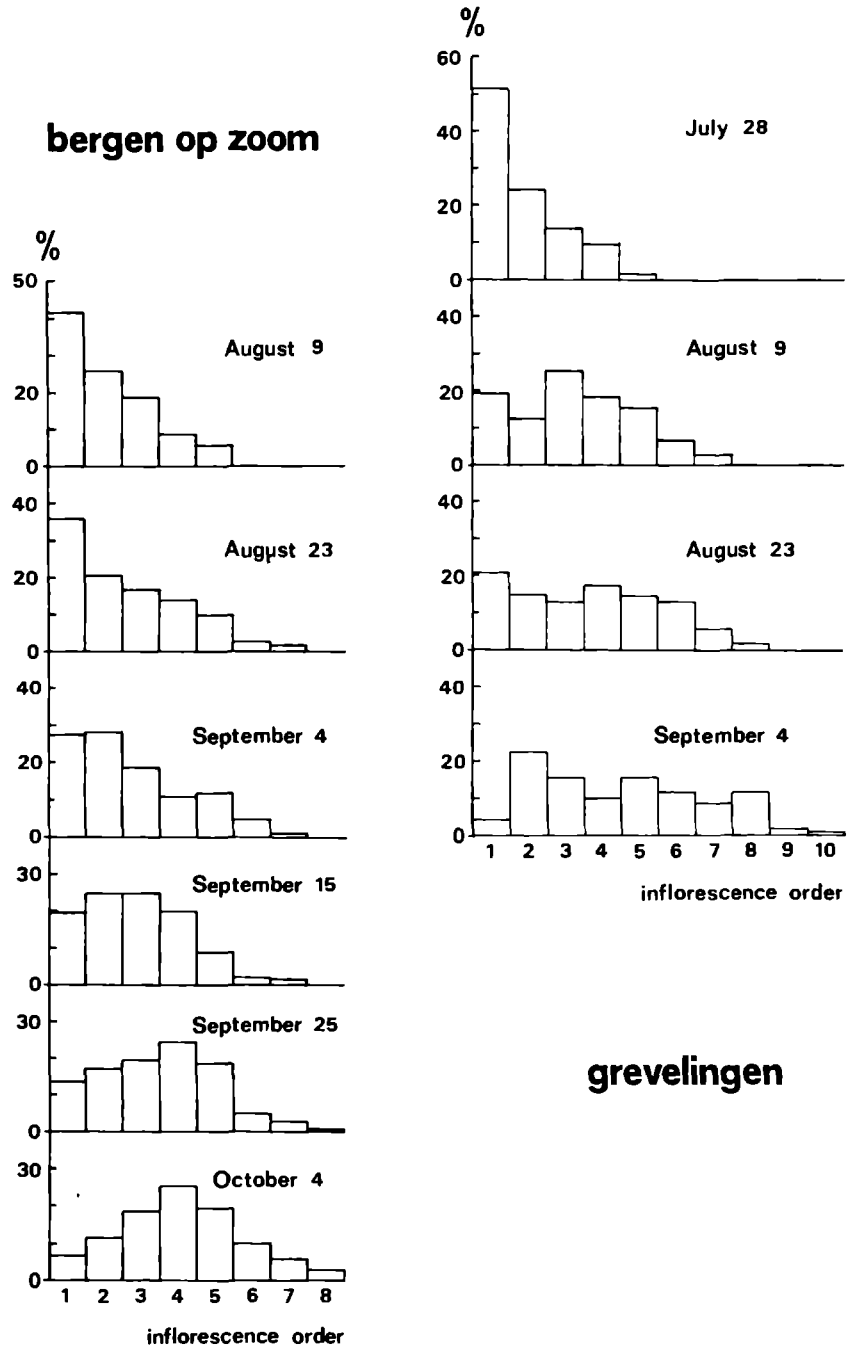
3. Course of flowering in the intertidal zone near Bergen op Zoom

The population near Bergen op Zoom that was studied, grows in the intertidal zone and is thus exposed to strongly varying conditions. At high tide the plants are always covered by a layer of water with relatively constant temperature, but at low tide the plants lay in very shallow pools in which great changes in temperature may take place. The temperature of the water in these pools is always close to the temperature of the air, which is generally between 15-25°C during the flowering season. Extreme values of about 10°C and 30°C may be reached during a cold night or a hot summer day respectively. A temperature rise of 5-6°C may take place within even one hour, at low tide. The flood water causes a temperature shock in the opposite direction. The water may cool down at night, especially at low tide. Flowering occurs under all of these temperature conditions.

Flowering started in 1975-1978 in July in the area examined, but then many of the flowering inflorescences were not fully developed. The lower flowers on the spadix were fully grown but towards the top the flowers were gradually less developed. In normal inflorescences there is a sudden transition between mature and undeveloped flowers. In many cases the spadix grew longer than the spatha and protruded for a part.

In order to study the course of flowering in the field, at different times in 1977 a number of flowering shoots was collected and the flowering state of at least 250 rhipidia was determined as follows. The lowest inflorescence of each rhipidium, which develops first, got the number 1, the next of the same rhipidium number 2 and so on, in the same way as Churchill & Riner (1978) did. From each rhipidium examined the number of the flowering inflorescence was noted, or, if no flowering occured at the moment of examination, the number of inflorescences

Fig. 4. Complete time course of flowering of one fertile shoot with four rhipidia in the aquarium. ♀ = start of erection of the styles; ♂ = dehiscence of anthers. Asterisks indicate the days between the start of erection of the styles and dehiscence of the anthers of the same inflorescence.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September 4
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September 15
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September 25
30
0
October 4
1 2 3 4 5 6 7 8
inflorescence order
%
60
40
20
0
July 28
40
20
0
August 9
40
20
0
August 23
40
20
0
September 4
1 2 3 4 5 6 7 8 9 10
inflorescence order
grevelingen

that had flowered Rhipidia of which the first inflorescence still had to start flowering and rhipidia completely off flowering, were not studied. In this way a picture was obtained from the state of flowering at the day of collecting (Fig. 5). To visualize the whole course of flowering during the season and to make a quick comparison to other areas easier, the average number of the flowering inflorescences per rhipidium of each collecting day was calculated and expressed graphically (Fig. 6).

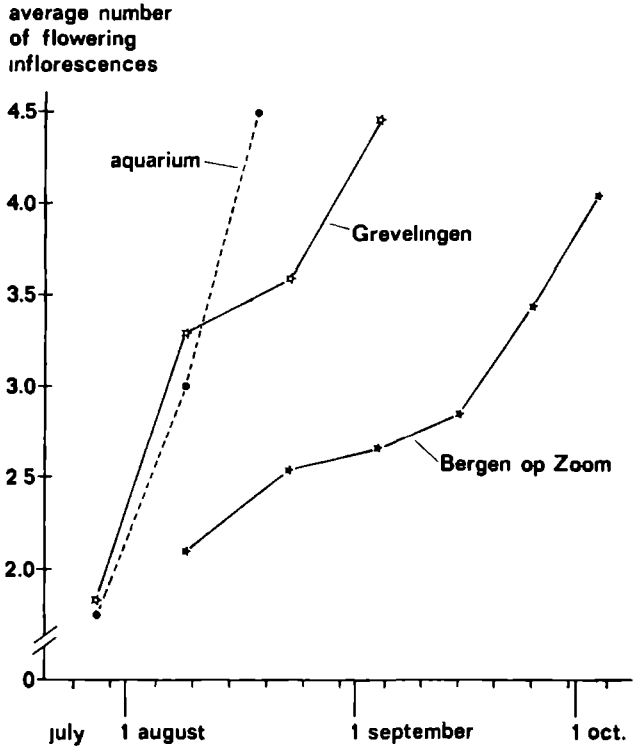

Fig. 6. Course of flowering of the population near Bergen op Zoom and the Grevelingen in 1977. For every date the average order number per rhipidium of the flowering inflorescences was calculated. For comparison a part of the course of flowering of the shoot of Fig 4 is drawn (period March 31-April 22).

Fig. 5 State of flowering of two populations near Bergen op Zoom and in the Grevelingen on different days in the flowering season of 1977. From at least 250 rhipidia on each day the order number of the flowering inflorescences was determined. The relative percentage of flowering inflorescences per order number is given.

←

The stages of the flowering inflorescences were noted: (1) styles projecting; (2) styles back, anthers not flowering; (3) anthers flowering and (4) pistils and anthers off flowering but next inflorescence not yet flowering (Table 1).

Flowering in this population stopped as the result of a decrease in the development of new fertile shoots, since the beginning of September. In the beginning of October only very few new fertile shoots were found. The shoots developed at most 8 inflorescences per rhipidium. In the middle of October nearly all plants were off flowering and withering. During the years that observations were made, slight differences concerning the end of the flowering season were found. In 1976 and 1978 still several flowering plants were noted on October 10 and 13 respectively and a clearly visible decrease in the number of flowering plants took place at the end of this month.

4. Course of flowering in the stagnant water of the Grevelingen

The plants of the population in the Grevelingen are continuously submerged. However, the fertile shoots grow so long that the main part is floating on the water surface. The water temperature is generally between 15-25°C during the flowering season. Sudden changes in temperature are absent.

Flowering starts here in the beginning of July. No incompletely developed inflorescences, as occurred near Bergen op Zoom, were noted at the beginning of the flowering season. From this population also flowering shoots were collected in 1977 and the state of flowering of at least 250 rhipidia was examined to follow the course of flowering in this area. The results are presented in Fig. 5. The averages

TABLE 1

Distribution of the flowering inflorescences over the different stages during the period of investigation. Percentages of the total number of investigated rhipidia. For comparison the relative duration of the flowering period (= stage 1 + 2 + 3) and the period between flowering of two successive inflorescences (= stage 4) of the flowering shoot of Fig. 4 is added.

Flowering stage of inflorescences	Bergen op Zoom	Grevelingen	Aquarium
1. styles projecting	6.1.	0.4	
2. styles back	48.8	55.5	59.3
3. anthers flowering	3.1	3.9	
4. off flowering	42.0	40.2	40.7

of the numbers of flowering inflorescences are shown in Fig. 6. The flowering stage of these inflorescences is given in Table 1.

Flowering in the population examined terminated much sooner than in the population near Bergen op Zoom. Already in the beginning of September the production of new fertile shoots stopped entirely and before the end of this month all plants were withering. In spite of this, at the end of October 1976 some flowering shoots ware washed ashore.

DISCUSSION

1. The development of the individual shoot during flowering

The development of the fertile shoots in the aquaria and in both populations examined differed somewhat from the development found by other investigators. Churchill & Riner (1978) found an increasing number of inflorescences per rhipidium acropetally along the shoot. Each branch was composed of one rhipidium and 4, rarely 5 rhipidia were developed. Jacobs & Pierson (1981) observed development and maturation starting at the base and proceeding to the apex of the shoot. They found a maximum number of branches of 6 and one of these branches, usually the first, could be composed of more rhipidia. The rhipidia of the fertile shoots in the aquaria flowered more or less synchronously (Fig. 4) and this was also true for the greater part of the flowering shoots in the field. Only rhipidia of large and several times branched fertile shoots showed some difference in their state of development. The inflorescences of the individual rhipidia, however, develop and flower one after an other and the flowering of two successive inflorescences is usually separated by a few days without flowering (Fig. 4). The whole fertile shoot enlarges by the development of the rhipidia. Although a rhipidium seems to be able to develop indefinitely, development stops after some time. The plants in the aquaria developed at most 9 inflorescences per rhipidium; the plants near Bergen op Zoom 8 and in the Grevelingen 10. Churchill & Riner (1978) mentioned an average of 3.2 inflorescences (max. 4) on the best developed (terminal) rhipidium. On the other hand Eicher (1875) found a maximum of 12. The limiting factors are probably of two kinds. On the one hand the development of new inflorescences may be terminated by unfavourable external conditions like low temperatures and lack of nutrients. On the other hand it is probable that the maximum number of inflorescences per rhipidium is genetically determined.

2. Course of flowering in the natural environment

In view of the long period that a fertile shoot may be flowering, one may suggest that there can neither be a real peak in flowering of *Zostera marina* in the field, nor a peak in the seed release. The phenological index, proposed by Phillips

(1976) is therefore hard to apply to this seagrass. Even if new flowering shoots are developed during a very short period of time, this will not be recognizable in the field as a peak in flowering as the same number of flowering plants will be present for an extended period. One may find such a peak (even if the production of new fertile shoots is prolonged) only by counting the number of flowering inflorescences of the different order numbers or by counting periodically the number of new fertile shoots on a certain surface area. Counting of new shoots has to be started before the beginning of flowering. A peak in the production of new fertile shoots, however, does not necessarily give a peak in flowering as fertile shoots not necessarily complete the development. Setchell (1929) found development of fertile shoots below a temperature of 15°C, but no flowering.

Flowering of a population may also stop when existing fertile shoots normally complete their development and get off flowering whereas the production of new shoots stops. This happened in the populations near Bergen op Zoom and in the Grevelingen at a different moment. The explanation is a complicated matter. There may be a more or less extended period of time between initiation and visible development of the fertile shoots. Most obvious controlling factors appear to be light, temperature and availability of nutrients. Light (daylength, irradiance) is a complicated factor as it interferes with ebb and flood in the intertidal zone. Effects of light and temperature are sometimes hard to observe separately as irradiance may cause rise in temperature, especially in shallow waters and during low tide. An important difference between the habitats of the populations examined is the presence of ebb and flood near Bergen op Zoom while the water of the Grevelingen is stagnant. It may be that the resulting temperature shocks cause a retarded development of the initiated fertile shoots near Bergen op Zoom. The actual stop of flowering may be caused by lack of nutrients, but this needs further research.

The flowering shoots, observed in the Grevelingen at the end of October 1976, are probably parts of plants growing in deeper water, where development may be retarded by the water temperature, which is slightly lower with less extreme values.

3. Differences between populations

Fig. 6 shows a remarkable difference in the average rate of flowering of the eelgrass near Bergen op Zoom and in the Grevelingen. This may be caused by difference in rate of flowering of the individual shoots and/or difference in the production of new shoots. Both parameters influence the angle of the graphs with the X-ordinate.

a. Rate of flowering of the individual shoots:

This may be calculated roughly from the histograms in Fig. 5. The front of the histograms of Bergen op Zoom moves between August 9 and 23 from inflorescence order number 5 to number 6 or 7. This means that the period of time between

the beginning of the flowering of two successive inflorescences is 7-14 days (average 10.5). This period can also be calculated from the shifting of the peak in the histograms. This peak becomes visible on September 4 at number 2 and is clearly recognizable at number 4 on September 25. So in 21 days the peak moves 2 order numbers, which gives an average period of 10.5 days between the beginning of the flowering of 2 successive inflorescences.

In the Grevelingen the front of the histograms moves between July 28 and September 4 from inflorescence order number 5 to number 9 or 10. This means a flowering period of 7.6-9.5 days (average 8.55) per inflorescence. A reliable peak is not to be distinguished with certainty in these histograms but if the 'peak' of number 1 on July 28 is the same as the little one of number 5 on September 4, going via number 3 on August 9 and number 4 on August 23, then one can calculate an average period of 9.5 days.

These calculations are rather rough but they indicate that the flowering rate is somewhat higher in the Grevelingen. For comparison: the average period between the beginning of flowering of two successive inflorescences of the flowering shoot of Fig. 4 was 8.6 days.

b. Production of new shoots:

An important factor determining the course of the graphs in Fig. 6 is the production of new fertile shoots. As the rhipidia of the same fertile shoot are generally in the same state of flowering in the plants of the populations examined, the production of new shoots may be concluded from the histograms in Fig. 5.

The histograms of Bergen op Zoom show that the production of new shoots increases first, reaching a maximum in the end of August and then decreases. This maximum is visible as a peak in the histograms after September 4. In the Grevelingen the production of new shoots increases at first and remains constant for an extended period of time or is slightly fluctuating. This is to be seen in the nearly equal amounts of inflorescences of the different order numbers since August 9. The production of new shoots suddenly stops at the end of August.

Both graphs of the Grevelingen and Bergen op Zoom in Fig. 6 show two kinks. The first one is probably the point when the first fertile shoots are getting off flowering. The second one is caused by a decreasing production of new shoots.

4. Relative duration of flowering stages in the field

The percentages of inflorescences in the different stages of flowering (Table 1) indicates the relative length of these stages. The low number of inflorescences with projecting pistils (stage 1) proves that pollination occurs very fast in both populations. This is naturally followed by a longer period in which the styles are back inside the spatha whereas the anthers are still present (stage 2). Also the duration of the dehiscence of the anthers is relatively short: less than 12 hours, if the total duration of stage 1 + 2 + 3 + 4 (= the period between the beginning of flowering of two

successive inflorescences) is 10 days. This is in accordance with earlier observations in aquaria (De Cock, 1980). There is a rather long period of time between the dehiscence of the anthers of one inflorescence and the projecting of the styles of the next one (stage 4). The relative duration of this period and of the period of flowering (stage 1 + 2 + 3) is the same in Bergen op Zoom, the Grevelingen and the aquaria. Thus the rate of flowering may be different but the relative duration of each stage is about the same.

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5. INFLUENCE OF LIGHT AND DARK ON FLOWERING IN *ZOSTERA MARINA* L. UNDER LABORATORY CONDITIONS

INFLUENCE OF LIGHT AND DARK ON FLOWERING IN *ZOSTERA MARINA* L. UNDER LABORATORY CONDITIONS

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ABSTRACT

De Cock, A.W.A.M., 1981. Influence of light and dark on flowering in *Zostera marina* L. under laboratory conditions. Aquat. Bot. (in press).

The female flowers of more than 80% of the inflorescences of *Zostera marina* L. flowered in the dark if they were kept in alternating periods of 12 h light and 12 h dark. This was independant of whether the dark period coincided with the solar day or night. No persistent endogenous flowering rhythm was found. The action of light seemed to be a fast acting photoinhibition which can not be extended over more than 24 h. Flowering can occur in constant light or constant dark but under the latter condition moulds and bacteria cause a premature stop.

Dehiscence of the anthers may also be influenced by periodic light but the sensitivity of anthers for alternating light and dark appeared to be varying during the flowering season. The influence of one or more unknown factors seems to be involved. A persistent endogenous flowering rhythm is absent. Flowering occurred normally in constant light and constant dark and anthers proved to be less sensitive to moulds and bacteria than the female flowers.

INTRODUCTION

Studies on the relation of flowering and light generally concern the induction of the development of reproductive organs. Marmelstein et al. (1968) reported photoperiodical control of flowering in the seagrass *Thalassia testudinum* Banks ex König. However, they concluded that photoperiod is more important to expression than to induction of flowering. McMillan (1976) studied the effects of salinity, temperature and daylength on the production of flowering stems in several seagrasses. He found that coincidence of these factors is probably critical to flowering and reproduction of *Halophila engelmanni* Aschers. but he suggested that temperature is the chief control of flowering period. Temperature also appeared to be important for flowering in *Cymodocea rotundata* (McMillan, 1979) but the role of light was insufficiently investigated in this study. The importance of light as well as temperature for flowering of the seagrass *Syringodium filiforme* Kütz. was demonstrated by McMillan (1980).

According to Setchell (1922, 1929) flowering of *Zostera marina* L. is restricted to the temperature range of 15-20°C and he suggested that temperature is the only

factor inducing development of flowering shoots in eelgrass. However, influence of light in this process was demonstrated by Backman & Barilotti (1976).

Induction of development of flowers is one aspect of the role of light in the flowering process; an other one might be an influence on flowering of mature inflorescences. While studying flowering of *Zostera marina* I found that the moment of anthesis of picked inflorescences varied during the day. There were problems in obtaining large amounts of flowering inflorescences of the same age for pollination experiments as especially during the day few inflorescences seemed to start erection of the styles. In view of the latter observation experiments were carried out to find whether there is a diurnal rhythm in flowering of pistils and anthers. An other question was: if such a rhythm exists, is it a persistent, endogenous rhythm or is it more or less instantly controlled by light and dark. Such an instant control makes manipulation of flowering possible and this might be a valuable help in further investigations of flowering, pollination and fertilization.

In this article I have used the term 'inflorescence' for the flowering unit of spadix, spatha and peduncle only and the term 'spathe' (spatha) if the spathal sheath and leaf are meant. For motivation: see De Cock (1981) (Chapter 4).

MATERIALS AND METHODS

Flowering shoots of *Zostera marina* plants were gathered in the intertidal zone near Bergen op Zoom (The Netherlands). Plants of this population are annual and flower abundantly. The material was transported to the laboratory in sea water. There the inflorescences were picked off and the spathal leaf blade was removed.

To test the influence of light on male and female flowers separately, two groups of inflorescences were selected:

a. Mature but not yet flowering inflorescences: for testing the influence of light on the erection of the styles;
b. Inflorescences with anthers still present but pistils projecting or bent back. The latter were used for testing the influence of light on the dehiscence of the anthers.

400 inflorescences of each group were distributed over 4 full-glass cisterns, with 1 l. synthetic sea water (Hw-Meeressalz, H. Wiegandt, Krefeld). The cisterns were covered with glass plates to avoid evaporation.

During the experiments all cisterns were kept at a constant temperature of 20°C. Water temperature was regularly checked and appeared to differ no more than 0.5°C. Light conditions were as follows:

a. constant light,
b. constant dark,
c. light during the day and dark during the night (periods of 12 hours each),
d. dark during the day and light during the night (periods of 12 hours each).

Of both series (male and female flowering) one cistern was kept under one of these

light conditions. The periods of 12 hours in these experiments coincided more or less with the natural day and night at the time of the experiments Source of light was a number of fluorescent lamps (Sylvania Powertube, cool white, F96T12/CW/VHO) at a light intensity of about 11,000 lux at the level of the water surface in the cisterns

The experiments were started in the evening, after the inflorescences had a period for adaption to the temperature room (in light) during 12 hours The number of flowering inflorescences was counted regularly after periods of 12 hours, in the morning and in the evening The time of counting coincided with the transfer of the cisterns from light to dark or vice versa An inflorescence was considered to be female flowering if at least one pistil had projected its style An inflorescence was considered to be male flowering if at least one half anther had dehisced. Flowering inflorescences were removed after every counting The experiments were extended until every inflorescence was flowering or until flowering obviously had stopped. Inspection of the cisterns in constant dark was carried out in green light of a very low intensity (less than 2 lux) Experiments were carried out three times in 1978 from July 27-Ausgust 2 (exp I), from August 5-11 (exp II) and from August 17-23 (exp. III) One experiment on male flowering, carried out in September 16-21 in 1977, is also presented for comparison

The course of female flowering during one period of 12 hours, after transfer to other light conditions, was examined in the same way as described above, except that during the 12 hours of the experiments, frequent checks were carried out. Flowering after transfer from light to dark was checked three times, flowering after transfer from dark to light seven times because of the low number of flowering inflorescences In each of these tests 100-150 inflorescences were present in the cisterns at the start of the experiment.

RESULTS

1 Flowering of the female flowers

In constant light 100% of the mature but not yet flowering inflorescences started flowering The course of flowering was quite regular. In the first period of 12 hours, however, flowering was clearly suppressed. No abnormal development of the female flowers was observed (Fig 1, 2)

In constant dark never a percentage of 100% flowering inflorescences was reached In the three replicates the final percentage was 10%, 82% and 42% respectively The water in the concerning cisterns smelled of H_2S after a few days and the inflorescences became mouldy. The course of flowering, however, was regular and showed no suppression in the first period. Also under this conditions no abnormal development of the female flowers was observed (Fig 1).

In both alternating light/dark regimes 100% flowering inflorescences was reached Flowering was not regular but differed in the successive periods the number

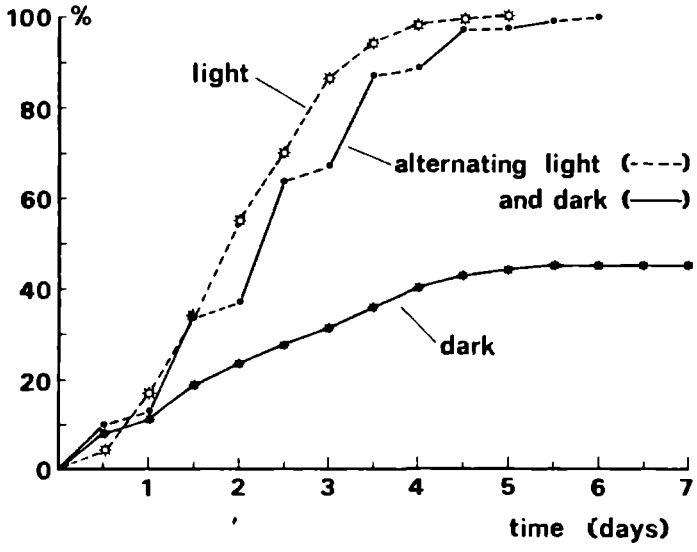

Fig. 1. Cumulative percentage of female flowering inflorescences in the course of time in constant light, constant dark and in alternating light/dark with the dark period during the night

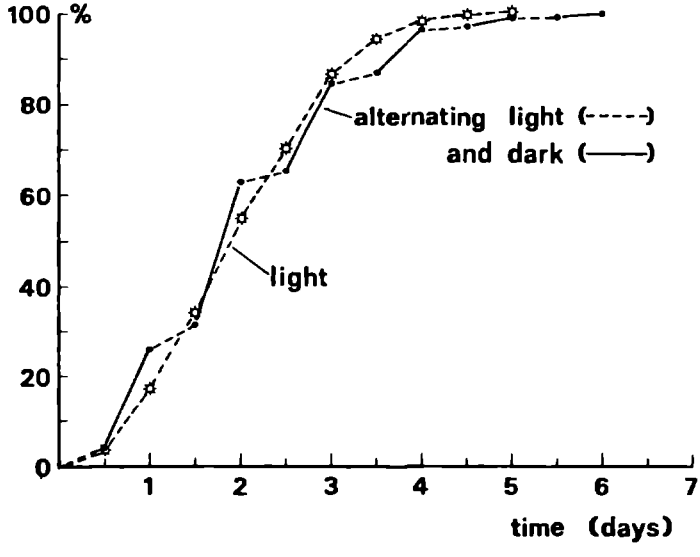

Fig. 2 Cumulative percentage of female flowering inflorescences in the course of time, in constant light and in alternating light/dark with the dark period by day.

of inflorescences that started flowering in the dark periods was significantly higher than that started in the light periods. This was already noticeable in the beginning of the experiments. In the cisterns that started in a dark period, a relative high number of inflorescences with projected pistils was found after the first twelve hours. In the cisterns that started in light, flowering was suppressed in the first period. The average flowering rate during the whole experiment was nearly the same of those in constant light and in alternating light and dark (Fig. 1, 2).

The results of the female flowering were similar in the three experiments under all conditions except in constant dark. The results in Fig. 1 and 2 are averages.

The course of flowering of the pistils during a light period was observed 7 times and during a dark period 3 times, with frequent checks. In a dark period flowering rate was constant or slightly increasing during the first 6 hours and became constant at a higher rate after that (Fig. 3). In constant light, flowering rate decreased rapidly and was 0 after 5 hours (Fig. 4).

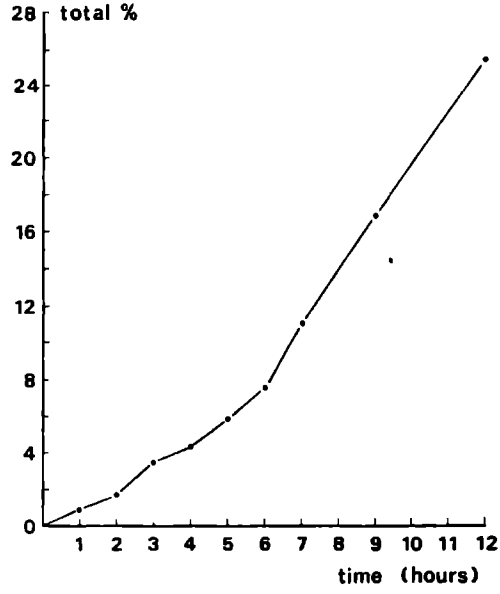

Fig. 3. Cumulative percentage of female flowering inflorescences in the course of time during a period of 12 hours in dark.

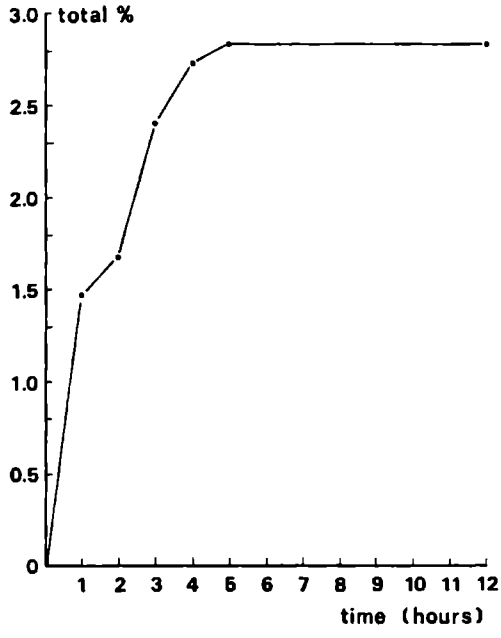

Fig. 4. Cumulative percentage of female flowering inflorescences in the course of time during a period of 12 hours in light.

2. Flowering of the male flowers (Fig 5)

Whereas the female flowers showed the same course of flowering under the same conditions in three replicate experiments, except in constant dark, the male flowers did not.

In constant light 100% of the inflorescences started male flowering but in experiment I (end of July) the number of flowering inflorescences was relatively high during the first period and decreased then gradually. In experiment II (be-

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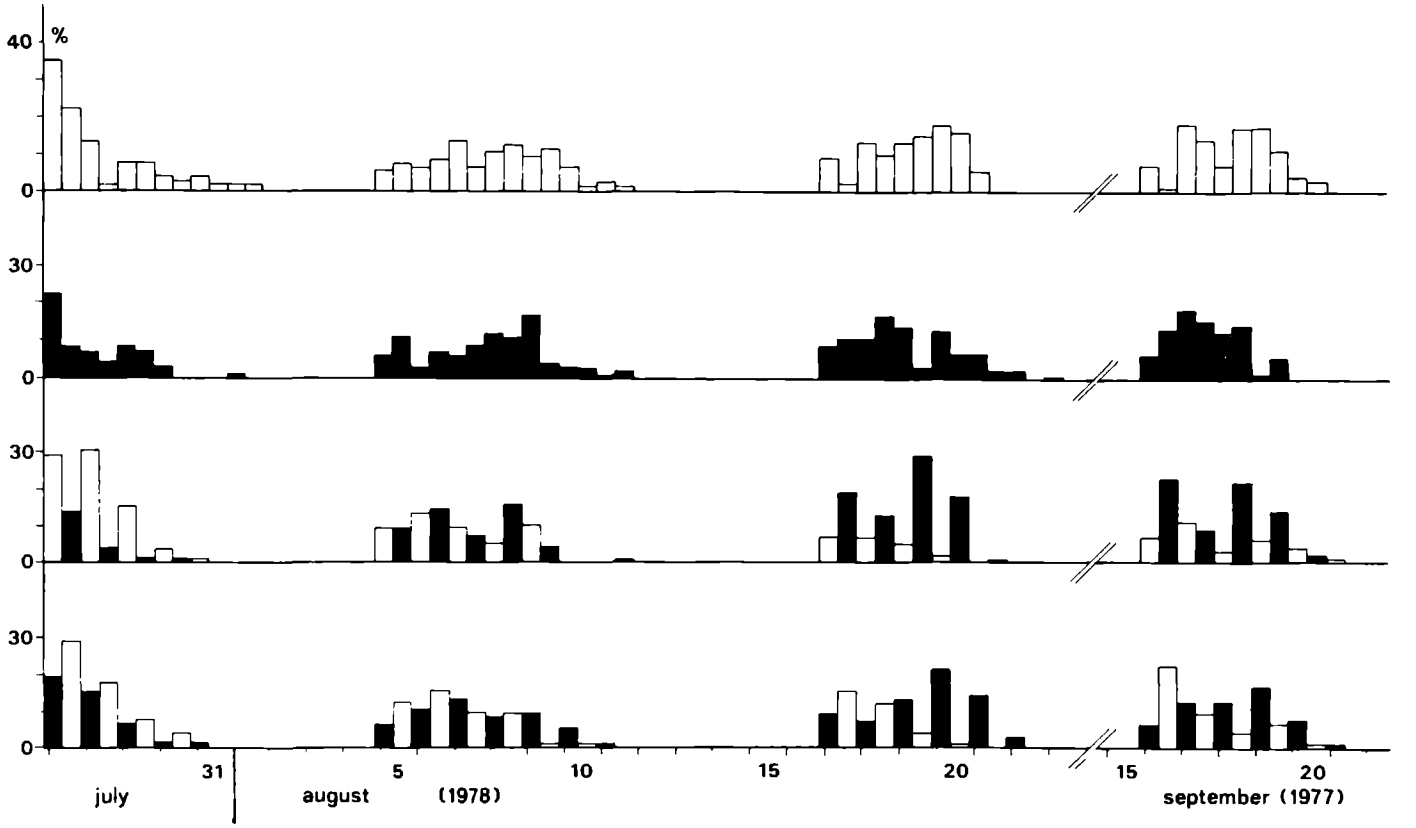
Fig. 5, Flowering of the male flowers in four experiments. From above to below: constant light, constant dark, alternating light/dark with dark period by day and alternating light/dark with dark period at night. Flowering in a light period is indicated by a white column; flowering in a dark period by a black column. The relative percentage of the total at the start of the experiment is given for each period of 12 hours. →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gin of August) and III (second half of August) the number was slightly fluctuating, without an obvious minimum or maximum.

In constant dark the number of male flowering inflorescences at the end of the experiments was 63, 96 and 97% respectively. The water in the cisterns was fouled by moulds and bacteria and the inflorescences were getting limp and slimy and finally sank to the bottom. The course of flowering was more or less similar to flowering in constant light.

In alternating light/dark periods the flowering percentage was always 100% at the end. However, the qualitative results in these tests differed greatly. In experiment I a significantly higher number of inflorescences showed anther dehiscence in the light periods. This was independant of the moment that this light period was given: during the solar day or night. However, the effect was more obvious if light was given during the night. The number of flowering inflorescences decreased gradually in the subsequent dark periods as well as in the light periods, as was the case under constant light conditions. The number of flowering inflorescences in the light periods, however, was always greater than in the preceding and following dark periods. In experiment II no influence of alternating light and dark was obvious, if the light period was given during the night. If the light period coincided with the solar day, the number of inflorescences with dehiscing anthers was somewhat higher in light during the first days but later on there seemed to be a shift of flowering towards the dark periods. However, this effect was not very clear in this experiment. In experiment III the rate of flowering was obviously higher in the dark periods in the cisterns with the light period during the night. If the light period coincided with the solar day, the rate of flowering was slightly higher in the light periods during the first two days, but significantly higher in the dark periods in the last four days. There appeared to be a shift of flowering from light to dark in this test: flowering in the subsequent light periods decreased whereas flowering in the dark periods increased.

DISCUSSION

1. Flowering of the female flowers

In a rhipidium only one inflorescence is flowering at the same time; when the anthers of an inflorescence have dehisced, the next one is still developing and starts flowering a few days later (4-5 days in sea near Bergen op Zoom) (De Cock, 1981). These developing but not yet flowering inflorescences were used for the test on female flowering. If the experimental conditions do not affect the flowering capacity, one may expect that these inflorescences flower within 4-5 days in equal daily numbers. This appeared to be the case under constant light conditions as well as in alternating light and dark. However, if the first experimental period was in light, then flowering was somewhat retarded in this period.

Erection of the styles of the inflorescences of *Zostera marina* is not liable to a persistent endogenous diurnal rhythm. This appears from the immediate synchronizing action of light under both alternating light/dark regimes and the very low number of flowering inflorescences in any of the light periods. A diurnal rhythm is caused by periodic light as is proved in the experiments, but it is only maintained by external stimuli.

The course of flowering in periodic light may be caused by a fast-acting photoinhibition as well as a photoactivation with an effect after 12 hours. There are several arguments for photoinhibition. The experiments were started in the evening, after the inflorescences had an adaption period in light for 12 hours. Before this adaptation period the light and temperature conditions were that of the natural habitat. If photoactivation occurs, which has its effect after 12 hours then a relative high number of flowering inflorescences in the first period of the experiments should have been the result, independant of whether this period was in light or dark. However, the number of flowering inflorescences was low in the cisterns which were kept in light during the first period and high in the cisterns in the dark. The course of flowering in a dark period (Fig. 3) and a light period (Fig. 4) make photoinhibition more likely too. In the case of photoactivation with 12-hours-later-effect, the increase in flowering rate would start in the beginning of a dark period. However, flowering rate is more than 0 during the first hours but only obviously higher after 5-6 hours since the beginning of the dark period. Moreover, the flowering rate decreases already in the first hour of a light period.

Action of light on flowering of the female flowers seems to be very fast but of a short duration. In constant light flowering is suppressed in the first period but constant after that. As an adaption period of 12 hours light preceded this first period, one may conclude that photoinhibition is not extended over more than 24 hours.

Flowering in constant dark appeared to be possible, even during extended periods of time. The varying and low final percentage of flowering inflorescences in the experiments is most probably due to growth of moulds and bacteria in the water and on the inflorescences.

2. Flowering of the male flowers

Anthers dehisce about 5 days after the pistils of the same inflorescence have projected their styles. Only inflorescences with projected or recurved styles in which the anthers were still present, were used for the test on male flowering. If the experimental conditions do not affect the capacity of flowering, one may expect that flowering of all inflorescences occurs within 5 days in equal daily numbers. This proved to be the case in experiment II and III and the experiment in 1977. However, experiment I showed a high number of flowering inflorescences in the first period and a decrease afterwards in each of the tested light regimes. As temperature and

light conditions were kept constant during the experiment, the cause of this decreasing flowering rate is probably to be found in the circumstances in the field before the material was picked off In the area where the material was collected, flowering starts in the second half of July, so this experiment was carried out in the early beginning of the flowering season An other possibility is the influence of an unknown, uncontrolled factor during the experiments

From the results in constant dark, it appears that light is not necessary for flowering Contrary to female flowers, the anthers seem less sensitive for growth of moulds and bacteria

It is obvious that periodic light may synchronize male flowering but the different results in the four experiments under alternating light/dark conditions may indicate that the sensitivity of anthers for periodic light is varying during the flowering season It is not clear by which factors this varying sensitivity is caused and in which way it may function in flowering in the field

The shift in flowering from light to dark periods in particularly experiment III and the experiment in 1977 suggests interference with one or more unknown, uncontrolled factors However, this shift was only noticeable if the light period coincided with the solar day and not if light was given during the night In experiment I no such an interference was obvious anther dehiscence in the light periods was higher since the start under both light/dark regimes For this reasons a persistent endogenous flowering rhythm seems also unlikely

Summarizing it may be concluded that light acts in male as well as in female flowering The highly synchronizing action of periodic light on erection of the styles makes manipulation of female flowering for experimental purposes possible Regulation of male flowering is more complicated as the sensitivity of the anthers for periodic light is varying during the season A persistent endogenous flowering rhythm appears to be absent, however, a diurnal rhythm in flowering may be caused by alternation of light and dark One has to be careful in extrapolating results from experiments in the laboratory to the situation in the field, but it seems reasonable to suppose that inflorescences of *Zostera marina* are influenced by light and dark in the same way in their natural habitat However, several other factors, such as temperature and salinity, may interact with light there

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6. INFLUENCE OF TEMPERATURE AND VARIATIONS IN TEMPERATURE ON FLOWERING IN *ZOSTERA MARINA* L. UNDER LABORATORY CONDITIONS

INFLUENCE OF TEMPERATURE AND VARIATIONS IN TEMPERATURE ON FLOWERING IN *ZOSTERA MARINA* L. UNDER LABORATORY CONDITIONS

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ABSTRACT

De Cock, A.W.A.M., 1981. Influence of temperature and variations in temperature on flowering in *Zostera marina* L. under laboratory conditions. Aquat. Bot. (in press).

Flowering of male and female flowers of *Zostera marina* L. has been observed in constant light under 5 temperature conditions: constant 15°C, 20°C and 25°C and alternating 15/20°C and 20/25°C. The rate of female flowering was higher at constant 20°C than at constant 15°C or 25°C. Alternation of temperatures led to a higher number of flowering inflorescences in the periods with the lower temperature.

The rate of male flowering increased with increasing, constant temperature. At alternating temperatures the number of male flowering inflorescences was not significantly different in the periods with the lower and the higher temperature. The average flowering rate was in these cases nearly the same as the flowering rate at the highest temperature.

INTRODUCTION

The seagrass *Zostera marina* L. grows in places where changes in temperature are rather limited in comparison to the habitats of land plants. This is particularly the case for plants, growing in the subtidal zone. Populations of the intertidal area are more exposed to temperature fluctuations. Nevertheless, Setchell (1929) found an important relation between temperature and life history even for plants in deeper water. According to this author no growth occurs below 10°C; vegetative growth takes mainly place between 10°C and 15°C and generative reproduction is restricted to water temperatures between 15°C and 20°C. Above 20°C generally a heat rigor sets in, which is irreversible. Higher temperatures cause the death of the plants. He suggested that no photo-periodism existed in this plant and that temperature was the only factor inducing flowering (Setchell, 1922).

Though these temperature intervals may be roughly valid for most of the subtidal populations, they are not for every population (Cottam & Munro, 1954; Riggs & Fralick, 1975) and they are certainly not valid for annual plants of intertidal areas. In the latter areas temperature may show very large fluctuations. Particularly in summer the sunshine may cause temperatures, considerably higher than 20°C and during the night relative low temperatures may occur. These effects are most obvious

during low tide. Annual populations keep their viability and flower abundantly under these conditions. The question may be stated whether temperature and variations in temperature, as they daily occur in the intertidal zone, influence flowering of *Zostera marina* and if so, in which way.

In this study the influence of a constant temperature of 15°C, 20°C and 25°C was examined as well as the effect of alternating temperatures of 15/20°C and 20/25°C. The temperature of 20°C was chosen as an intermediate temperature because this seems to be a good approximation of the temperature of sea water in summer in the area where the examined plants grew.

In this article I have used the term 'inflorescence' for the flowering unit of spadix, spatha and peduncle only and the term 'spathe' (spatha) if the spathal sheath and leaf are meant. For motivation: see De Cock (1981a) (Chapter 4).

MATERIALS AND METHODS

Flowering shoots of *Zostera marina* plants were gathered in the intertidal zone near Bergen op Zoom (The Netherlands). Plants of this population are annual and flower abundantly. They are completely submerged at high tide and during low tide they are laying in very shallow pools in which great changes in temperature may take place. Handling and selection of two groups of inflorescences in a different stage of development for separate examination of flowering of pistils and anthers, was the same as described by De Cock (1981b).

500 inflorescences of each group were distributed over 5 full-glass cisterns with 1/2-1. synthetic sea water (Hw-Meeressalz, H. Wiegandt, Krefeld). The cisterns were covered with glass plates to avoid evaporation.

During the experiments all cisterns were kept in constant light as this proved to give a regular course of development of the flowers, except in the first experimental period of 12 hours following after a period for adaptation in light (De Cock, 1981b). Source of light was a number of fluorescent lamps (Sylvania Powertube, cool white, F96T12/CW/VHO), which gave a light intensity of about 11,000 lux at the level of the water surface in the cisterns.

Temperature conditions were as follows:

a. constant 15°C.
b. constant 20°C.
c. constant 25°C.
d. alternating 15°C and 20°C in periods of 12 hours.
e. alternating 20°C and 25°C in periods of 12 hours.

Of both series (male and female flowering) one cistern with 100 inflorescences was kept under one of these temperature conditions. Different temperatures were achieved by using water baths. If alternating temperatures were tested and a cistern was transferred from one bath to another, the temperature of the sea water adapted to the new temperature within 15 minutes.

The experiments were started in the evening after a period for adaption in light for 12 hours. The number of flowering inflorescences was counted regularly after periods of 12 hours. These countings coincided with the transfer of the cisterns concerned to an other temperature. An inflorescence was considered to be female flowering if at least one pistil had projected its style. An inflorescence was considered to be male flowering if at least one anther had dehisced. Flowering inflorescences were removed after every counting. Experiments were discontinued when the maximum percentage of flowering inflorescences was reached. All experiments were carried out in duplicate at different times.

RESULTS

1. Flowering of the female flowers (Fig. 1, 2)

The morphological features of flowering were not altered by the different temperature conditions. Flowering showed a regular course at all of the three constant temperatures (Fig. 1). The rate of flowering was about the same at 15°C and 25°C but clearly higher at 20°C. Flowering was suppressed in the first 12 hours period at 20°C, even longer at 15°C and noticeably longer at 25°C.

Abnormal flowering was observed at 25°C. Simultaneous flowering of pistils and anthers occurred after 3 1/2 day at that temperature (Fig. 1). These inflorescences were counted, however, together with the normal, female flowering inflorescences. A few inflorescences started flowering with deshiscence of the anthers. These were not involved in the countings and had to be removed as it could not be seen whether the styles were still immature inside the spatha or back again after (self-) pollination. For this reason the final percentage of flowering inflorescences could not reach 100% at 25°C.

The course of flowering in the cisterns with alternating temperatures was quite 'irregular' (Fig. 1, 2). Under both temperature regimes the number of flowering inflorescences was higher in the periods with the lower temperature. This was most obvious if alternating periods of 20°C and 25°C were given but also evident in a 15/20°C regime. The average flowering under both alternating temperature regimes was intermediate between the rates at the corresponding constant temperatures. Table 1 shows the cumulated percentages of inflorescences, flowering in the periods with the same temperature, for each of the cisterns at alternating temperatures.

2. Flowering of the male flowers (Fig. 3, 4)

After a slight suppression in the first period, flowering was rather regular at both constant and alternating temperatures. Under the constant temperatures the flowering rate of the anthers proved to increase with increasing temperature: the highest flowering rate was reached at 25°C (Fig. 3).

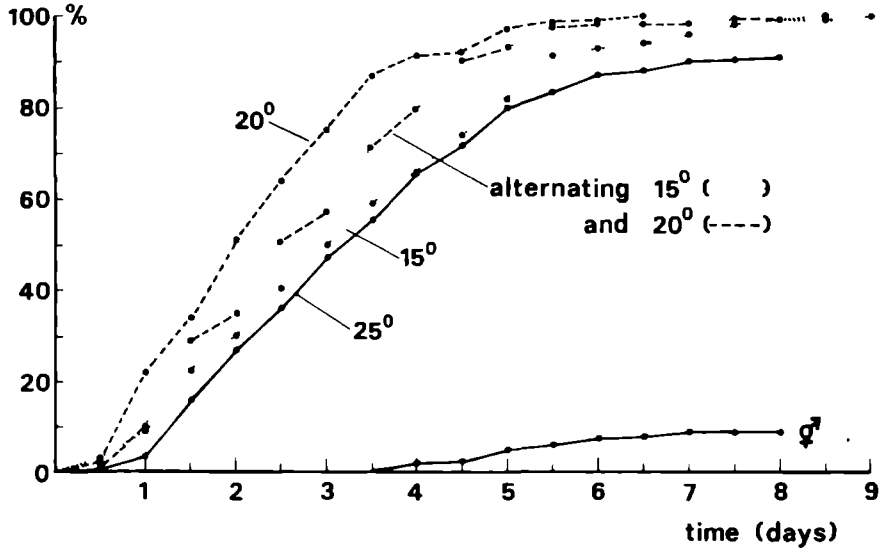

Fig. 1. Cumulative percentage of female flowering inflorescences in the course of time at a constant temperature of 15°C, 20°C and 25°C and at alternating 15/20°C and the cumulative percentage of inflorescences with simultaneous flowering of pistils and anthers at constant 25°C.

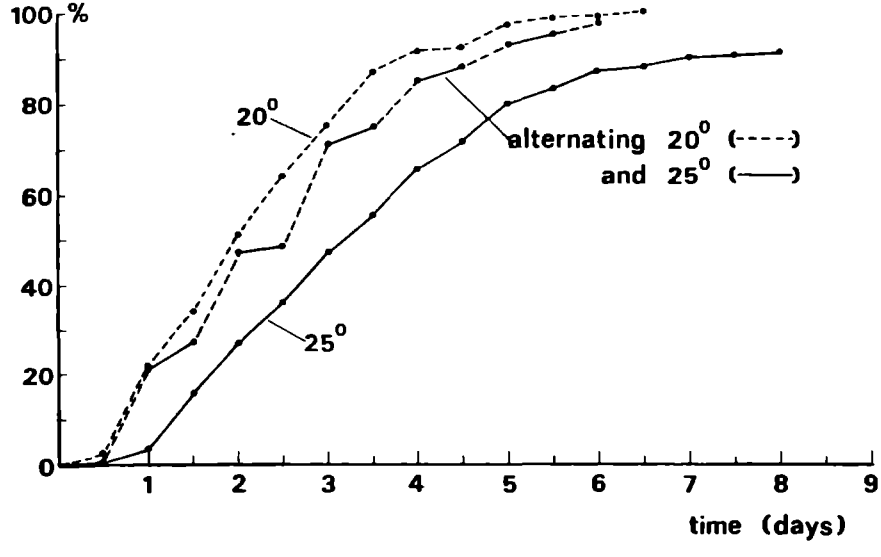

Fig. 2. Cumulative percentage of female flowering inflorescences in the course of time at a constant temperature of 20°C and 25°C and at alternating 20/25°C.

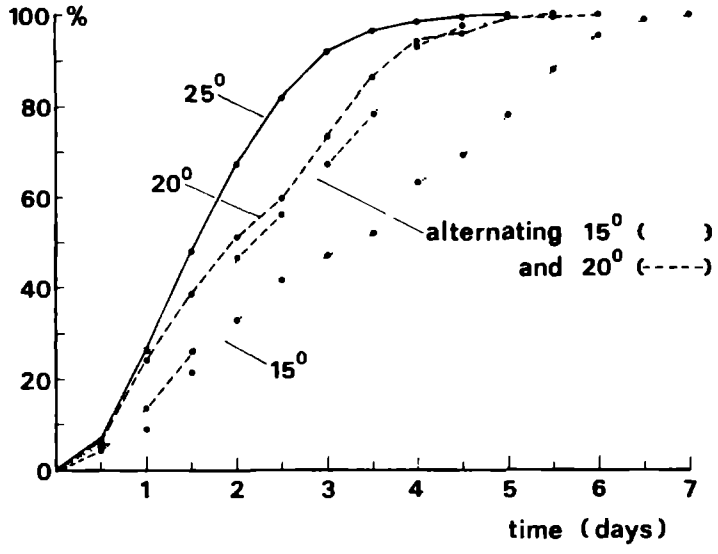

Fig. 3. Cumulative percentage of male flowering inflorescences in the course of time at a constant temperature of 15°C, 20°C and 25°C and at alternating 15/20°C.

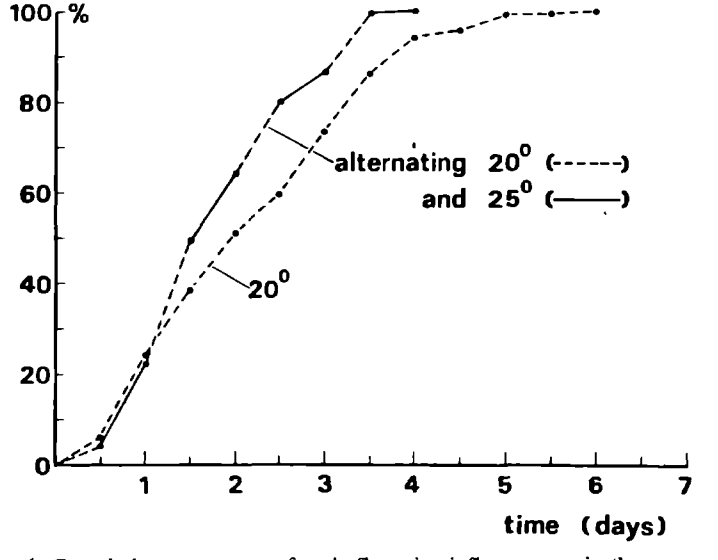

Fig. 4. Cumulative percentage of male flowering inflorescences in the course of time at a temperature of constant 20°C and alternating 20/25°C. The graph of the flowering course at constant 25°C is not drawn as it coincided with the graph of alternating 20/25°C.

TABLE 1

Results at the alternating temperature regimes. The cumulated percentage of inflorescences, flowering in the periods with the same temperature is given for male and female flowering.

	female flowering		male flowering	
temperature regime →	15/20°C	20/25°C	15/20°C	20/25°C
flowering at 15°C	66.7	--	57.8	--
20°C	33.3	80	42.3	55
25°C	--	17.5	--	45

Alternating temperatures of 15/20°C or 20/25°C did not result in alternating periods with higher and lower rate of flowering; the total number of flowering inflorescences was somewhat higher in the periods with the lower temperature (Table 1). Throughout the period of investigation, the average flowering rate was the same (20/25°C) or nearly the same (15/20°C) as the flowering rate at the higher corresponding constant temperature, thus 25°C and 20°C respectively.

DISCUSSION

1. Flowering of the female flowers

The experiments were started after the inflorescences had an adaptation period in light for 12 hours. The suppression of flowering in the first experimental period must be due to the experimental light conditions (constant light). However, this photoinhibition is only present during about 24 hours in constant light at 20°C (De Cock, 1981b). So the longer suppression, in the second experimental period at constant 15°C and 25°C, can be attributed to temperature.

It appears that there is an optimum in temperature for female flowering near 20°C. Flowering starts earlier and proceeds faster at 20°C than at 15°C or 25°C.

Not only different constant temperatures but also changes in temperature influence female flowering. It is quite remarkable that transfer from 20°C to 15°C induces a higher flowering rate, though a temperature of 20°C seems favourable for flowering, if given constantly. It is nog clear whether a lower temperature stimulates or a higher temperature suppresses.

2. Flowering of the male flowers

Contrary to the female flowers, the male flowers did not have an optimum in the investigated temperature range. Flowering increased with increasing temperature. There is no early period of suppression of flowering at one of the three constant temperatures: differences in the graphs are only caused by differences in flowering rate, not by a short term suppression as occurs in female flowering. As alternation of temperatures did not lead to differences in flowering rate, the influence of temperature on the anthers seems to be a stimulation of the development only and not a factor in the diurnal course of flowering. This is supported by the phenomenon of simultaneous flowering of pistils and anthers at 25°C in the test for female flowering. This is obviously caused by a suppression in the first periods and a lower flowering rate of the female flowers at this temperature, as well as the higher rate of development of the anthers.

3. Temperature effects in the intertidal zone

For the populations of *Zostera marina* in the intertidal zone, particularly the effect of alternating temperatures on flowering is important. Water temperature is influenced here by two interfering factors: day and night on the one hand and high and low tide on the other. The temperature regime depends on the time of the day when flood and ebb occur. Moreover, as also light has a great influence on flowering of male as well as female flowers (De Cock, 1981b), one may not consider the temperature factor alone. It is most likely that temperature and light co-operate in some way. In the case of flowering of the female flowers it may be expected that the lower temperature during the night intensifies the effect of the darkness, resulting in a higher flowering rate. Higher temperatures during the day may co-operate with light in suppressing flowering of the female flowers.

ACKNOWLEDGEMENTS

The author wishes to thank Prof. Dr. H.F. Linskens for his stimulating interest and Dr. A.F. Croes for critically reading the manuscript. Thanks are also due to Prof. Dr. C. den Hartog for his valuable comments and for correcting the English text.

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SAMENVATTING

SAMENVATTING

Het bleek mogelijk om *Zostera marina* planten uit zaad te kweken in een aquarium. Onder de testomstandigheden (temperatuur van ca. 18°C en daglengte van 16 uur) kwamen de planten ook in bloei (Hoofdstuk 1).

Er bleek geen verschil waar te nemen in bloeigedrag van afgebroken bloeistengels, losse inflorescenties en bloeiende planten in het aquarium. Het bloeiproces begint met het oprichten der stijlen: de stijlen buigen tot ze een rechte hoek vormen met het vruchtbeginsel. Het uiteinde met de stempel raakt daarbij buiten de schede en kan bestoven worden. De stijlen blijven in deze toestand (stadium 1) tot bestuiving optreedt. Pas na bestuiving buigt de stijl terug tot ze binnen de schede is (stadium 2). Het terugbuigen begint 3-7 uur na de bestuiving. Ongeveer na 7 uur of nog later breekt de stempel af. Tussen 1 en 7 dagen na het oprichten van de stijlen bloeien de (half-)antheren. Dit proces wordt aangekondigd door vorming van gasbelletjes binnen de schede. Er vindt zijdelingse afplatting van de thecae plaats en het vrije uiteinde buigt omhoog. Intussen raakt de vasthechting aan de spadix steeds verder los. Deze plaats is waarschijnlijk de bron van de gasbelletjes. De theca-wand begint dorsaal aan de top te openen en de wanden krullen naar buiten. Daarbij worden de schederanden, die na het terugbuigen der stijlen weer gesloten waren, opnieuw geopend. Vervolgens komt het pollen vrij in het water. Het openen der antheren is in de natuurlijke volgorde het derde stadium van de bloei. Het vierde stadium is de rijping van de zaden. Na de bestuiving groeit de zaadknop uit tot het hele vruchtbeginsel wordt gevuld. Vruchtbeginsel en zaadknop groeien samen verder en na ongeveer een maand (gemiddeld 32,9 dagen in het aquarium) volgt het vijfde stadium: het vrijkomen der rijpe zaden. Dit vrijkomen geschiedt door het openen van de vruchtwand, langs een dorsale naad. De beide vruchtwandhelften krullen vervolgens naar buiten en voor de derde maal worden de schederanden opzij geduwd. Daarna richt het vrije uiteinde van de vruchtwand zich op en duwt het zaad naar buiten. Daarna volgt de 'verwelking'. Van verwelking in de zin van verkommering van de kroonbladeren is hier geen sprake omdat de kroon ontbreekt. Het bladachtig deel van de spatha valt vaak vroegtijdig af, meestal tijdens de zaadvorming, soms eerder. Enkele dagen voor het vrijkomen van de zaden wordt de groene kleur van de rest van de spatha geelgroen. Vervolgens verandert deze kleur via geel en bruin naar bijna zwart in enkele weken tijds. In de aquaria bleven de bloeistengels vastzitten en in zee worden de stengels soms losgewerkt, vermoedelijk door waterstromingen.

Bestuiving kan zowel geschieden via het wateroppervlak als onder water. Als een anthere onder water opent komt het pollen vrij en verspreiden de draden zich geleidelijk en los van elkaar door het water. Indien ze echter aan de oppervlakte openen, dan verspreiden de draden zich met een snelle beweging in een netwerk over de oppervlakte. Verder verspreiding wordt bemoeilijkt doordat het netwerk overal aan vast plakt. Opvang door de stempels is een passief proces; aktieve

omstrengeling door de pollendraden werd niet waargenomen. Bestuiving in zee bleek vrij efficiënt te geschieden. De vrouwelijke bloempjes bleken grotendeels bestoven te zijn binnen 24 uur na het oprichten der stijlen. Pogingen om meer van het bestuivingsproces te weten te komen met behulp van kunstmatige bestuivingsexperimenten, mislukten doordat het percentage effektief bestoven stampers bij identiek uitgevoerde proeven variëerde van 0 tot 100% (Hoofdstuk 2).

Korte uitsteekseltjes van 5-10 µ werden aangetroffen op pollendraden die enige tijd in natuurlijk zeewater hadden gelegen, of in verdunningen daarvan. Deze uitsteekseltjes werden zelden waargenomen bij pollen in kunstmatig zeewater. Wel werden deze korte pollenbuisjes waargenomen in kunstmatig zeewater waaraan 10% saccharose en een extrakt van de vrouwelijke bloempjes was toegevoegd. Deze toevoegingen hadden een stimulerend effekt op de groei van de pollenbuisjes, die ontstaan waren in natuurlijk zeewater, tot een maximale lengte van ongeveer 50 µ. (Hoofdstuk 3).

De ontwikkeling van de bloeistengel werd bestudeerd bij aquariumplanten. De inflorescenties van een rhipidium ontwikkelden na elkaar; gelijktijdige bloei van twee inflorescenties van hetzelfde rhipidium werd niet waargenomen. Tussen twee opeenvolgende inflorescenties is doorgaans een periode van enkele dagen zonder bloei, dat wil zeggen, de oprichting van de stijlen van de jongere inflorescentie vindt pas plaats enkele dagen nadat de antheren van de oudere gebloeid hebben. Maximaal 6-9 inflorescenties per rhipidium werden gevormd bij de aquariumplanten. Bij de opeenvolgende inflorescenties viel een afname te konstateren van de lengte van de pedunculus en de spatha en van het aantal bloempjes per spadix. De verschillende rhipidia van dezelfde bloeiende scheut ontwikkelden vrijwel synchroon. Alleen bij grote, vaak vertakte bloeistengels trad enig verschil op.

De ontwikkeling van de bloeistengels in de getijdezone nabij Bergen op Zoom en in een ondiep gedeelte van de Grevelingen bleek iets langzamer te gaan dan in het aquarium. Bij Bergen op Zoom nam de produktie van nieuwe bloeischeuten toe vanaf het begin van de bloei in juli tot aan het eind van augustus, waarna een geleidelijke afname plaats vond. Begin oktober stopte de nieuwvorming geheel en aan het eind van die maand was bijna elke plant aan het verwelken. In de Grevelingen werd een toename van de produktie van nieuwe scheuten waargenomen in juli. Daarna was de nieuwvorming vrijwel konstant tot begin september, toen een plotselinge stop optrad. De gemiddelde bloeisnelheid is waarschijnlijk iets hoger in de Grevelingen dan in het onderzochte gebied bij Bergen op Zoom (Hoofdstuk 4).

Licht bleek vooral op de bloei van de vrouwelijke bloempjes een sterke invloed te hebben. Bij afwisselend 12 uur licht en 12 uur donker bloeiden de vrouwelijke bloempjes van bijna 80% van de inflorescenties in de donkere perioden. Invloed van een persistent endogeen dag/nacht ritme werd daarbij niet gevonden. Waarschijnlijk is hier sprake van remming door licht maar dit remmend effekt verdwijnt als de inflorescenties langer dan 24 uur in het licht zijn. In konstant licht trad dan ook na aanvankelijke remming, normale bloei op. Ook in konstant donker trad

bloei op, maar slechts een gedeelte der inflorescenties raakte in bloei, als gevolg van aantasting door bakteriën en schimmels. De invloed van licht op de mannelijke bloei bleek te variëren gedurende het seizoen. Waarschijnlijk is daarbij nog een onbekende faktor in het spel. Een endogeen dag/nacht ritme werd niet gevonden. Bloei vond ook plaats in konstant licht en konstant donker. De antheren bleken minder gevoelig voor aantasting door schimmels en bakteriën dan de vrouwelijke bloempjes (Hoofdstuk 5).

Ook temperatuur bleek van invloed op het bloeiverloop. Bij 20°C bleek de vrouwelijke bloei sneller te verlopen dan bij 15°C of bij 25°C, als deze temperaturen konstant werden gegeven. Bij afwisselend 15/20°C en 20/25°C bleek de bloeisnelheid echter groter in de perioden met de lagere temperatuur. De bloei van de antheren had geen optimum bij de drie geteste konstante temperaturen: hoe hoger de temperatuur, hoe hoger de bloeisnelheid was. Bij afwisselende temperaturen werd geen verschil gevonden in bloeisnelheid in de perioden met de hogere of lagere temperatuur. De gemiddelde bloeisnelheid was echter nagenoeg gelijk aan de snelheid bij de hogere temperatuur, als die konstant werd gegeven (Hoofdstuk 6).

CURRICULUM VITAE

Arthur Wilhelmus Antonius Maria de Cock werd op 5 februari 1950 te Tilburg geboren. In dezelfde plaats doorliep hij de lagere school en volgde hij middelbaar onderwijs aan het Paulus Lyceum. In juni 1967 behaalde hij het diploma h b.s -B.

In september 1967 begon hij met de studie biologie aan de Katholieke Universiteit te Nijmegen. Het kandidaatsexamen werd afgelegd in januari 1971. Voor het doktoraalexamen werd een bijvak Geschiedenis van de Biologie bewerkt onder leiding van Dr. P. Smit en een bijvak Botanie onder leiding van Prof. Dr. H F. Linskens. Op de afdeling Zoologie I (hoofd Prof. Dr. J.M. Denucé) werd een hoofdvak bewerkt, onder leiding van Dr. F.S Lukoschus. Tijdens het hoofdvak werd door hem enkele malen geassisteerd bij de prekandidaatskursussen 'Histotechniek' en 'Histologie' (leiding Dr. F.S. Lukoschus) In het schooljaar 1971/1972 gaf hij biologieles aan het Cobbenhagencollege te Tilburg. Na het doktoraalexamen, waarvoor hij slaagde in juni 1974, volgde een aanstelling als wetenschappelijk medewerker aan de afdeling Botanie I van de Katholieke Universiteit voor de bewerking van een promotieonderwerp. Deze aanstelling duurde tot december 1978. Gedurende deze jaren assisteerde hij enkele malen bij de prekandidaatskursus 'Ontwikkelingsgeschiedenis en Systematiek van de Cryptogamen' (leiding Dr. Ir. G. van den Ende).

In 1975 trad hij in het huwelijk met Johanna Catharina Jacoba Maria Flipsen. Uit dit huwelijk werd in 1976 zoon Maurice geboren en in 1977 dochter Chantal.

Stellingen

I

Bij het bloei- en bestuivingsproces bij *Zostera marina* L. spelen eb en vloed en de ermee samenhangende verschijnselen een hoofdrol.
Dit proefschrift

II

De konklusie van Rasmussen dat de "wasting disease" van het zeegras in de dertiger jaren primair werd veroorzaakt door verhoogde watertemperaturen, volgt niet uit zijn onderzoek.
Rasmussen, E., 1977. in: McRoy, C.P. & Helfferich, C. (eds), Seagrass Ecosystems, a scientific perspective. Marine Science 4 (D.W. Hood ed.). Marcel Dekker Inc. - New York & Basel.

III

Het funktioneren van de mens in de natuur wordt ten onrechte als onnatuurlijk beschouwd.

IV

Bij een beschrijving van een nieuwe mijtesoort verdient een tekening de voorkeur boven een goede foto.

V

Bij onderscheiding van genotypen en endospermmutanten bij maïskorrels met behulp van interferentiemikroskopisch onderzoek van het pericarp, dienen naast de reliëfvorm en -hoogte, zo mogelijk, ook andere kenmerken betrokken te zijn, zoals de afmetingen van de cel.
Linskens, H.F., Pfahler, P.L. & De Cock, A.W.A.M., 1974.
Theor. appl. Genet. 45: 137-139.
Pfahler, P.L., Linskens, H.F. & De Cock, A.W.A.M., 1975.
Euphytica 24: 53-58.

VI

De gebruikelijke indeling van artikelen in inleiding, materiaal en methode, resultaten, diskussie, samenvatting, leidt tot een starheid die de kwaliteit van de publikatie kan aantasten.

VII

Het verdient aanbeveling om aan een wetenschappelijke publikatie na de diskussie een hoofdstuk "Ideeën en suggesties voor verder onderzoek" toe te voegen, indien de auteur het onderzoek zelf niet meer voortzet.

VIII

Het onderscheid tussen "kunst" en "kitsch" is "kunst"-matig.

IX

Het is voor zijn omgeving aangenamer dat iemand slapend rijk wordt dan dat hij stinkend rijk wordt.

A.W.A.M. de Cock

Nijmegen, 17 december 1980